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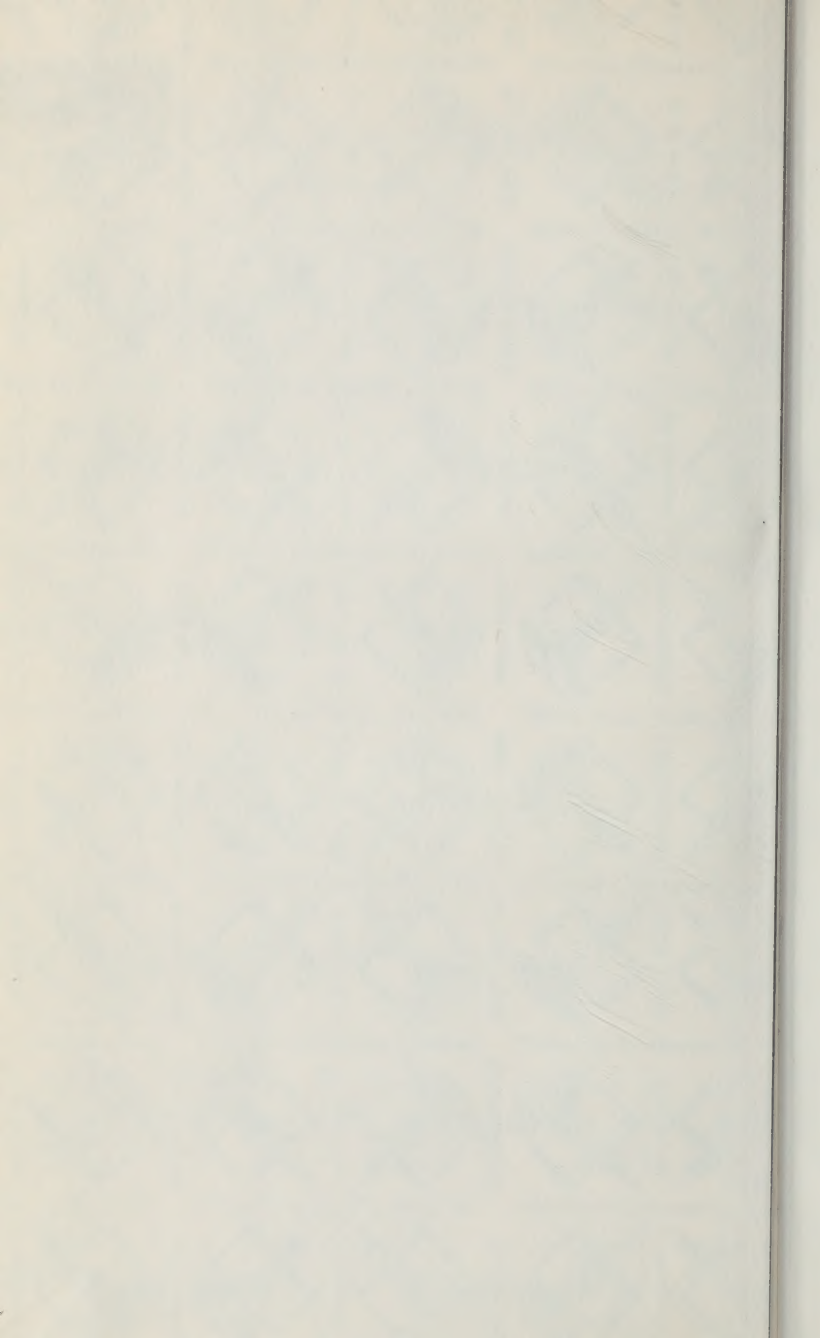


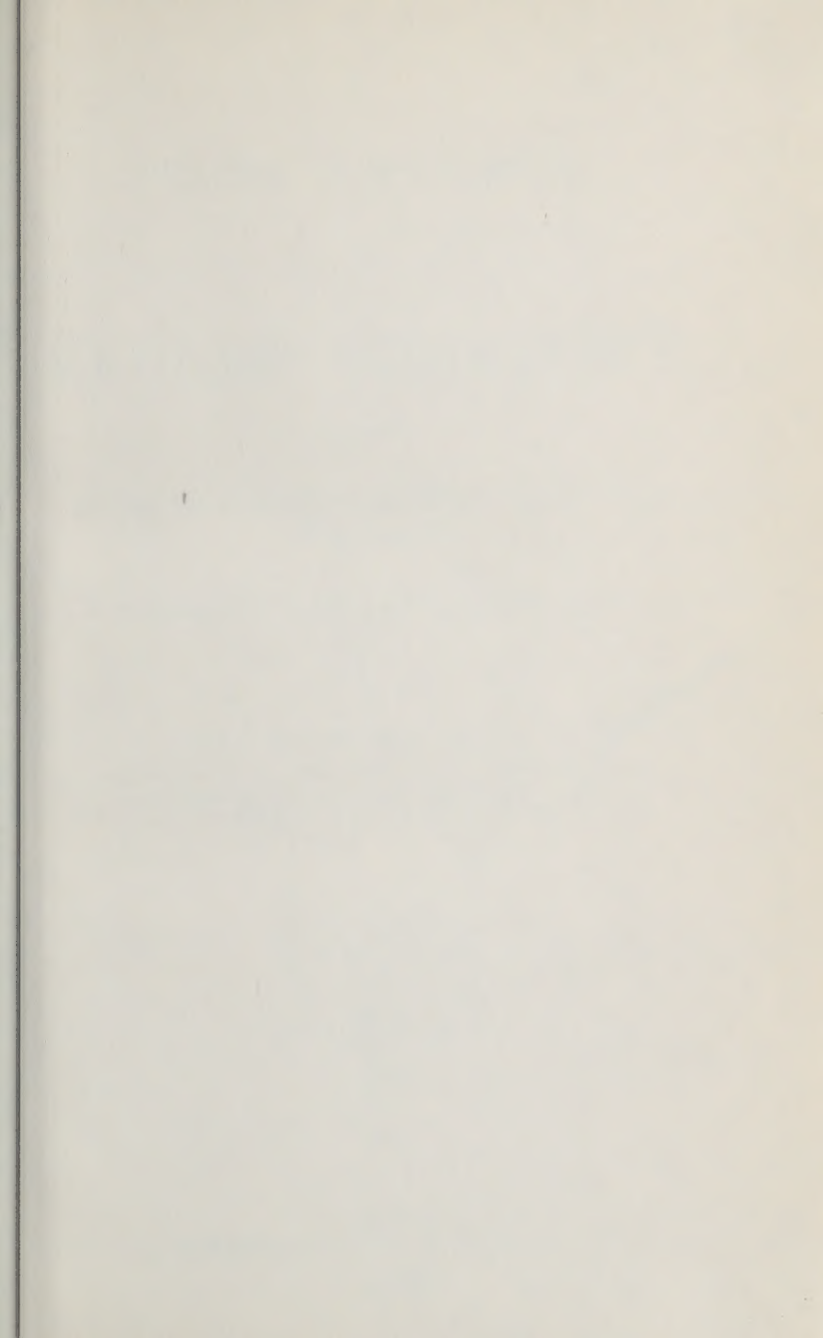
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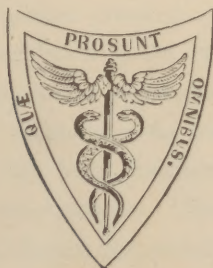
A
PRACTICAL HANDBOOK
OF
MEDICAL CHEMISTRY
APPLIED TO
CLINICAL RESEARCH AND THE DETECTION
OF POISONS.

PARTLY BASED ON "BOWMAN'S MEDICAL CHEMISTRY."

BY

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PREFACE.

As a rule, the occupation of a practising physician precludes the possibility of his being a chemist; and the errors which have crept into the history of physiological chemistry through hasty conclusions from imperfectly performed work, are sufficiently numerous to demonstrate that chemical research can only be undertaken by one whose time may be wholly devoted to the subject. The chemistry of life is as yet in a rudimentary condition: with few exceptions, it consists of a mass of unconnected facts, more or less developed as the interest of the experimenter has been arrested.

Until the metamorphoses of the proximate principles of organized matter can be accomplished in the laboratory in a manner which may be comparable to their transformations in the body, zoochemistry cannot take the position of an exact science. The results of recent studies on the more obscure fermentations have indicated that these phenomena may have an important bearing on biological chemistry; and the synthesis of complex compounds closely related to the albuminoid bodies, serves

to show that we may hope for a better understanding of the vital functions at a not very distant day.

But while biological chemistry is unsatisfactory as a science, an acquaintance with those compounds which normally form part of our tissues, the variations which these compounds undergo in altered conditions of the health, and an ability to detect substances which are themselves the evidence of pathological action, are often of incalculable service to the physician, in confirming or guiding diagnosis, and consequently in selecting treatment.

At one time, Bowman's little book on Medical Chemistry supplied the practical information required by physicians in the application of chemistry to clinical research; but Bowman's Medical Chemistry has long been out of print, and newer and better methods have arisen with the development of chemistry; at the same time, no suitable text-book embracing these methods has appeared in English.

While, therefore, it has not been deemed advisable to follow the general plan of the former work, a plan which at the present day is open to many serious objections, this little book is designed to fill the place so well filled by the other before its time of usefulness had passed.

The only two works on zoochemical analysis which have any claim to a scientific basis, are those of Gorup-Besanez, and of Hoppe-Seyler. On these, this book has in part been modelled, and it is believed that the arrangement will be found acceptable and convenient. The matter

is presented in as few words as possible for its clear explanation, and the discussion of doubtful chemical questions, always perplexing except to professional chemists, as well as the description of processes which cannot be recommended, are as a rule entirely omitted, as are also those crude methods of approximate estimation which amount to little more than conjectures, and are entirely untrustworthy.

The first part of the book is devoted to a brief description of the proximate principles which take part in normal and pathological vital action, and the properties by which they may be separated from their associate compounds, and identified.

In the second part, the composition of the more important liquids and solids of the body is considered, together with the processes by which they may be analyzed, both for the estimation of their normal constituents, and for the detection of compounds whose presence must be regarded as pathological. The methods given in this part have been carefully selected as those which will yield the most accurate results in hands not specially skilled in chemical manipulations. All of these operations require practice and great care if any degree of accuracy be desired; without this, analysis can be of but little service.

The third portion of the book treats of the detection of the more ordinary poisons. A toxicological investigation is seldom undertaken by a physician, but cases may sometimes arise in which a comparatively easy pre-

liminary examination may indicate either the importance of a subsequent analysis by an expert chemist, or that suspicions of poisoning had been groundless. The directions here given will be found sufficient in such cases. This is the only part of the work which is modelled on the plan of Bowman's Chemistry.

WM. H. GREENE.

UNIVERSITY OF PENNSYLVANIA,
1st March, 1880.

CONTENTS.

	PAGE
INTRODUCTION	13
<i>Manipulation.</i> Solution, extraction, filtration, dialysis.	
Evaporation, desiccation, incineration	13-16

PART I.

ORGANIC PROXIMATE PRINCIPLES TAKING PART IN THE ANIMAL ECONOMY.

FATTY ACIDS	17
Formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid	17-19
Detection and separation of the fatty acids	19
LACTIC ACID, paralactic acid, zinc lactate and paralactate, de- tection of lactic acid	20
OXALIC ACID,—calcium oxalate	22
SUCCINIC ACID, detection in the urine	24
BENZOIC ACID	25
GLUCOSE, detection by Moore's test, Trommer's test, Fehling's test, Böttger's test	27-29
INOSITE, separation from muscular juices and from urine, tests for inosite	29
LACTOSE, decomposition by acids	30
GLYCOGEN, differences from starch	31
CHOLESTERIN, extraction from biliary calculi	32
<i>Stercorin or serolin, excretin</i>	33
UREA, action of acids and alkalies, chlorine, hypochlorites, hypobromites, mercuric nitrate. Extraction from urine	34
<i>Urea nitrate, urea oxalate</i>	35-36
Detection of urea	37
HIPPURIC ACID, preparation from urine	37

	PAGE
URIC ACID, detection by the murexide test,—extraction from small quantities of liquid	39-41
XANTHINE, detection in urinary calculi	42
HYPOXANTHINE, compounds of silver nitrate with xanthine and hypoxanthine	43
<i>Guanine</i>	44
CARNINE, preparation from extract of meat	44
OXALURIC ACID, extraction from urine	45
CREATINE, preparation from flesh,—decompositions	46
CREATININE, its compound with zinc chloride,—detection in urine	48
LEUCINE, preparation and properties	50
TYROSINE, reactions by which it is distinguished from leucine	52
NEURINE OR CHOLINE, extraction and properties	53
TAURINE, preparation from ox-gall	54
GLYCOCHOLIC ACID, Pettenkofer's test for biliary acids	56
TAUROCHOLIC ACID, containing sulphur, is easily distinguished from glycocholic acid	57
CHOLIC ACID, a decomposition product of the two preceding acids	58
LECITHINE, extraction from yolk of egg	59
<i>Phosphoglyceric acid</i>	59
CYSTINE, detection in urinary calculi	60
ALBUMINOID BODIES, general properties, composition, behavior with reagents	61
<i>Detection of an albuminoid body of undetermined nature</i>	63
<i>Classification of albuminoid bodies</i>	64
Albumen, nitric acid test, phenol test, metaphosphoric acid test,—egg albumen, estimation of albumen	65-66
<i>Vitellin, globulin, hydropisin, pancreatin, paralbumen, metalbumen</i>	67
<i>Hemoglobin, oxyhemoglobin, reduced hemoglobin, absorption spectra</i>	68-70
<i>Hematin, hematin hydrochloride or hemin. Hematoidin</i>	70-71
<i>Fibrin, fibrinogen and fibrinoplasmin</i>	71
<i>Myosin, preparation from muscles</i>	72
<i>Syntonin, preparation from various forms of albumen</i>	73
<i>Albuminose or peptones</i>	73
<i>Casein, alkaline albuminates</i>	74
<i>Animal ferments, ptyalin, pepsin, pancreatic ferments</i>	74-76
SUBSTANCES RESEMBLING ALBUMINOID BODIES, general composition	76
<i>Ossein, gelatin, chondrin, keratin, mucin</i>	77

	PAGE
ANIMAL PIGMENTS, melanine	78
<i>Bilirubin</i> , Gmelin's test for biliary pigments, <i>biliverdin</i> , <i>bilifuscin</i> , <i>biliprasin</i> , extraction from bile and separation	79-81
<i>Hydrobilirubin</i> , existence in febrile urine	82
<i>Indican</i> , chemical properties, detection in urine	83
<i>Indigotine</i> , identical with uroglauцин, urocyanin, etc.	84
<i>Indirubin</i> , extraction from violet urine	85

PART II.

ANALYSIS OF SECRETIONS, EXCRETIONS, ETC.

URINE	87
Physical properties	87
Normal constituents	88
Abnormal constituents, accidental constituents	89
<i>Chemical examination</i> , quantity, consistence, specific grav- ity, reaction, cause of normal acidity, alkaline urine, color	90-94
<i>Detection of abnormal substances—</i>	
Albumen, nitric acid test	94
Glucose, application of Fehling's test	95
Inosite	96
Lactic acid, found after poisoning by phosphorus	97
Biliary acids and pigments, Pettenkofer's test, Gmelin's test, red hepatic urine, blue and violet urine	97-100
Cystine, urine smells of hydrogen sulphide	100
Leucine and tyrosine, separation and detection	101
Blood	102
Ammonia	103
<i>Rapid qualitative analysis of urine</i>	103
<i>Quantitative analysis</i> , estimation of normal constituents.	
Approximate composition	103-104
Estimation of water and of fixed matters, method of Magnier de la Source, Neubauer's approximate cal- culation	105
<i>Organic constituents—</i>	
Estimation of urea by Liebig's method, preparation of the mercuric nitrate solution, corrections	106-109
Yvon's method by sodium hypobromite	110
Esbach's method	113
Estimation of uric acid	114
Estimation of hippuric acid	115
Estimation of creatinine	116
<i>Mineral constituents—</i>	
Sodium chloride, volumetrically by silver nitrate	117
Phosphoric acid, by uranium acetate	119
Sulphuric acid; Vogel's approximate method	121
<i>Abnormal constituents—</i>	
Estimation of albumen	122
Estimation of glucose by Fehling's solution	123
Estimation of ammonia	125

URINARY SEDIMENTS, unorganized sediments, organized sediments, extraneous matters	125-134
URINARY CALCULI, method of analysis,—uric acid calculi,—ammonium urate calculi,—xanthine,—cystine,—albuminoid and epithelial matters,—calcium oxalate,—calcium and magnesium phosphates,—earthy carbonates,—mixed calculi	134-139
BLOOD, general physical properties,—normal chemical constituents,—abnormal constituents,—general chemical properties	140-142
<i>Analysis of blood</i> ,—detection of urea,—uric acid,—creatinine and creatinine,—glucose,—mineral salts,—biliary acids and pigments,—leucine and tyrosine,—ammonia,—carbon monoxide	143-146
<i>Quantitative analysis</i> ,—average composition of human blood,—method of analysis, and calculation of results,—estimation of water and of fixed matters,—mineral salts,—fibrin,—calculation of results	146-153
analysis of serum,—estimation of albumen, salts, etc., Hoppe-Seyler's method,—estimation of hemoglobin,—anatomy of blood	153
<i>Detection of blood spots and stains</i> ,—microscopical examination,—detection of hemoglobin and of hematin by aid of the spectroscope,—detection by the production of hemin crystals,—confirmatory tests	161-167
ANALYSIS OF SEROUS LIQUIDS	167
<i>Quantitative analysis</i>	169
<i>Special serous effusions</i> ,—pleural effusions,—hydrocele,—expectorations after thoracentesis	170
PUS, general properties,—analysis,—blue pus, pyocyanin,—microscopic appearance of pus	172-174
EXTRACTS OF THE MUSCULAR TISSUES,—separation of creatine, fatty acids, lactic acid, inosite, uric acid,—Neubauer's method	175-178
JUICES OF GLANDULAR ORGANS,—Staedler's method of analysis	179
BONE, TEETH, AND BONY STRUCTURES,—general chemical composition,—quantitative analysis	181-186
SALIVA,—detection of potassium sulphocyanate,—ptyalin, salivary concretions	187-189
GASTRIC JUICE,—estimation of acidity and of hydrochloric acid	190-192
BILE,—general properties	193
<i>Analysis of biliary calculi</i>	194

	PAGE
MILK.—General properties and composition,—analyses of cream.—Quantitative analysis of milk.—Millon and Commaille's method.—Chevalier and Henry's method.—Baumhauer's method.—Volumetric estimation of lactose.—Rapid estimation of butter by the lacto-butyrometer.—Lehmann's method.—Various analyses of milk.—Milk of different animals	196–208
Examination of commercial milk,—detection of adulterations,—use of the lacto-densimeter	209
Diseased milk	211
Colostrum	212
SPERMATIC FLUID,—detection of seminal stains	214
EXCREMENTS, general composition	217
ANALYSIS OF THE ASH OF ANIMAL SUBSTANCES—	
Qualitative analysis	219
Quantitative analysis,—estimation of potassium and sodium, phosphate of iron, calcium, magnesium, sulphuric acid, chlorine, phosphoric acid	223–225

PART III.

ON THE DETECTION OF POISONS.

Precautions to be taken in chemico-legal investigations	227
ARSENIC, usual forms in which it occurs	229
Identification of pure arsenious oxide	230
Detection in organic mixtures	232
Destruction of organic matter	233
Detection of cupro-arsenical pigments	235
Reinsch's test	236
Marsh's test	237
Electrolytic test	241
Quantitative estimation	243
ANTIMONY, most common form tartar emetic,—reactions of tartar emetic,—tests for antimony	244
Separation of antimony from arsenic, and quantitative estimation	246
TRIN, differences from arsenic and antimony	247
MERCURY, separation from organic mixtures,—reactions of mercury,—electrolytic test, and quantitative estimation	248–252
LEAD, separation from organic mixtures,—examination of water suspected to contain lead,—quantitative estimation	253–256
COPPER, reactions and quantitative estimation	257–260
ZINC	261

	PAGE
DETECTION OF ACIDS, general method	262
<i>Sulphuric acid</i> ,—sulphate of indigo	264
<i>Hydrochloric acid</i> ,—quantitative estimation	265
<i>Nitric acid</i>	266
Detection of mineral acids in stains on clothing	267
<i>Oxalic acid</i>	268
HYDROCYANIC ACID, detection in a state of vapor,—detection in solution,—quantitative estimation	269-272
PHOSPHORS, Mitscherlich's method,—Fresenius's method	273
DETECTION OF ALKALOIDS, Stas's process,—general reagents	275-277
<i>Conine</i>	278
<i>Nicotine</i>	279
<i>Morphine</i> , Usler and Erdmann's process,—T. G. Wormley's process,—chemical tests for morphine,—for meconic acid	280-284
Opium alkaloids other than morphine	284
<i>Strychnine</i> , Rodger and Girdwood's process	285
<i>Brucine</i> , physiological tests	287
DETECTION OF ALCOHOL IN ORGANIC MIXTURES	288
CHEMICAL EXAMINATION FOR THE DETECTION OF A POISON THE NATURE OF WHICH IS UNKNOWN	289
Volatile poisons,—alkaloids,—metallic poisons	289-292

APPENDIX.

VOLUMETRIC ANALYSIS, normal solutions and standard solutions	293-295
PREPARATION OF NORMAL SOLUTIONS, normal sodium carbonate, normal sulphuric acid, normal nitric acid, normal sodium hydrate	295-296
ESTIMATION OF CARBONIC ACID GAS, FREE AND COMBINED, IN WATER	297
ESTIMATION OF AMMONIA	299
ESTIMATION OF COMBINED ACID IN NEUTRAL SALT	299
STANDARD SOLUTIONS.—Objects attained by definite strength of standard solutions in analysis of urine,—solution of mercuric nitrate for estimation of urea,—silver solution for estimation of sodium chloride,—uranium solution for estimation of phosphates	300-302
WEIGHTS AND MEASURES	303

MEDICAL CHEMISTRY.

INTRODUCTION.

Manipulation.

§ 1. A FAMILIARITY with general chemical manipulation and the processes usually followed in analysis, is absolutely necessary to one who would study with profit the complex constituents, secretions, and excretions of the human body.

The processes followed in physiological chemistry are essentially the same as those adopted in inorganic chemistry, but the nature of the substance under examination frequently demands special modifications of these processes. Such methods of procedure will be indicated when considering their applications; it will be sufficient for the present to mention certain precautions necessary in the more ordinary operations of physiological analysis.

SOLUTION. EXTRACTION. FILTRATION. DIALYSIS.

The solution of a solid is always greatly facilitated by dividing the substance as finely as possible. If it be hard, it may be pulverized; but soft substances, such as flesh, glandular structure, etc., must be cut up finely with a knife or scissors, and, if further division be necessary, it may be effected by trituration with coarsely powdered glass or quartz sand.

Various solvents are employed, water, alcohol, ether, chloroform, benzol, acids, and alkalies, being used, according to the nature of the body under examination. One

or the other of these solvents often permits the separation of a body in a comparatively pure state from substances which may be soluble in other liquids.

When the action of the liquid upon the substance is but partial, and leaves certain substance undissolved, the process is called *extraction*. It is better to employ a small quantity of the solvent at a time, and when this is completely saturated to filter or decant the solution, treating the residue with a fresh portion of the solvent, and repeating the operation until all of the soluble matter is removed.

It is frequently necessary to submit a substance to the successive action of several solvents.

When rapid filtration is required, a folded filter may be used, but when the precipitate or coagulum must be removed from the filter, the folds would frequently occasion a serious loss of substance. The filtration of many animal liquids is extremely slow and difficult, unless a filter-pump or aspirator be employed.

Dialysis is frequently not only the most expeditious, but the sole, method by which an uncrystallizable body may be separated from a body which will crystallize. A good dialyzer may be easily made by removing the bottom from a bottle, and replacing it by a sheet of parchment paper bound tightly over the edges. It should be suspended in a vessel of water so that the paper bottom does not touch the bottom of the vessel. If a solution of albumen or gum Arabic mixed with crystallizable salts be introduced into such a dialyzer, the salts will gradually pass through the diaphragm, leaving the uncrystallizable substances in the bottle. The water in the exterior vessel should be frequently changed, and the dialysis is rendered much more rapid by occasionally agitating the fluid in the bottle.

EVAPORATION. DESICCATION. INCINERATION.

The evaporation of most animal liquids may be conducted on the water bath; there are but few organic substances of animal origin which are not destroyed or altered by a temperature above 100°. It is advanta-

geous that the capsule should be as flat as possible, and that its sides should be heated above the level of the liquid it contains: the creeping of the liquid on the sides of the dish may be thus avoided.

Many substances will not support a temperature of 100° ; such must be evaporated in an air oven, or in a vacuum.

The object of desiccation is to remove from substances, even when they appear quite dry, the hygroscopic water which they contain, and which is obstinately retained by animal matters. Without this desiccation, the results of analysis by weighing would be almost valueless.

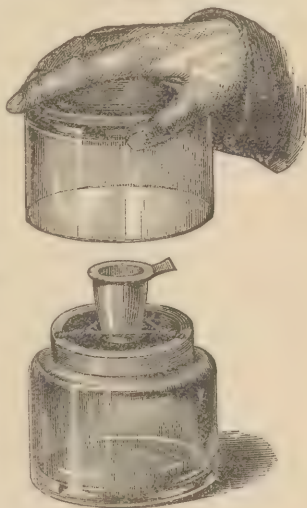
Desiccation is accomplished by the aid of a hot-air or water oven, or, in case the substance will not support a temperature of 100° , by exposing it for some time in a vacuum over either quicklime or strong sulphuric acid.

Should the substance be not very hygroscopic, the hot-water or steam oven may be employed; as the interior temperature of a hot-water oven generally falls several degrees below 100° , it is advisable to add a considerable proportion of glycerin to the water; by this means the temperature may be maintained at 100° , and as the glycerin does not volatilize with the water, it serves continually as the water is replaced.

However, when the substance is not decomposed by a temperature a few degrees above 100° , much more satisfactory results are obtained, and time is economized, by exposing it to a temperature of 105 – 110° in a hot-air oven.

After a substance has been dried, it must be placed over a dish containing sulphuric acid or quicklime, and

Fig. 1.



covered at once with a bell jar (Fig. 1), and so allowed to cool before weighing. Any other suitable form of desiccator may be used.

Incineration.—In order to determine the amount of inorganic salts contained in an animal product, it is necessary to incinerate the substance at a bright red heat until the whole of the carbon is burned out. Great care is required in the incineration of animal matters, as they usually increase enormously in volume when first heated, and frequently soften, so that there is danger that portions may be projected from the dish or crucible. The heat must, therefore, be applied slowly and cautiously until the matter is thoroughly carbonized: when this is accomplished there is no further danger, and the temperature may be raised and maintained until the incineration is complete, as is indicated by the white or very pale color of the ash.

The burning of animal matters generally produces most offensive gaseous products, so that incinerations should be performed under a hood connected with a good chimney.

PART I.

ORGANIC PROXIMATE PRINCIPLES TAKING PART IN THE ANIMAL ECONOMY.

Fatty Acids.

§ 2. The organic acids which may be extracted from the tissues, secretions, and excretions, do not, as a rule, exist there in the free state, but in the form of salts, either of the alkaline or earthy metals, or of organic radicals, in the latter case constituting organic ethers.

Of the fatty acids constituting the series $C^n H^{2n} O^2$, the first members up to the term $C^{10} H^{20} O^2$ (capric acid) have been found in the secretions of the skin and in the liquids of certain glands. All of these acids are liquid at ordinary temperatures, and volatile; they have peculiar penetrating odors; they are soluble in water, alcohol, and ether, but their solubility in water diminishes as their proportion of carbon increases, and in the same order their boiling-points are higher. They are all strongly acid, and with the bases form salts which are generally soluble and crystallizable.

The higher members of the series, palmitic and stearic acids, exist as glycerides in the animal fats. They are odorless and tasteless; solid at ordinary temperatures, and insoluble in water.

§ 3. FORMIC ACID, $CH^2 O^2$.—Salts of this acid are found in the sweat, and in the liquids of the spleen and pancreas, in the juices of the muscular tissues and the brain. Traces of it have been found in the urine.

It may be distinguished from all other acids of the series by its pungent odor, and by its reducing silver nitrate to metallic silver by the aid of heat. It likewise

reduces mercuric oxide when boiled with that compound. The alkaline formates are very soluble in water, and the other formates all more or less soluble. Ferric chloride produces a blood-red color in solutions of the neutral formates, and on boiling the liquid a yellow basic salt is precipitated.

§ 4. ACETIC ACID, $C^2H^4O^2$.—Acetic acid has been detected in the liquids of various organs, in the sweat and bile, and in the blood of lepers. It sometimes exists in the free state in the matters vomited by children, being formed by fermentation of farinaceous matters in the stomach.

Its characteristic odor is well known; like formic acid, it dissolves in water in all proportions, but unlike that acid, it is not decomposed when heated with concentrated sulphuric acid. Silver nitrate produces a white precipitate in strong solutions of the acetates; this precipitate of silver acetate dissolves in warm water, but separates in crystalline needles on cooling. Silver nitrate is not reduced by boiling with acetic acid. With ferric chloride, the acetates produce a red color, analogous to that yielded under the same circumstances by the formates.

Silver acetate contains 64.67 per cent. of silver.

§ 5. PROPIONIC ACID, $C^3H^6O^2$.—Propionic acid has been detected in the sweat and bile, and sometimes in the contents of the stomach. It has a penetrating odor, recalling at the same time that of acetic and that of butyric acids. It is quite soluble in water, but if calcium chloride be added to its solution, the acid separates, and floats on the surface of the liquid.

The propionates much resemble the acetates. Silver propionate contains 59.67 per cent. of silver.

§ 6. BUTYRIC ACID, $C^4H^8O^2$.—This acid exists in a free state in the perspiration, and sometimes in the contents of the stomach and in the urine. Butyrates are found in the muscular liquids, and in the fluids of certain glands.

Its odor recalls that of rancid butter, in which it was first discovered. It is soluble in all proportions of water, alcohol, and ether, but calcium chloride separates it from its aqueous solutions.

The alkaline butyrates are deliquescent, difficult to crystallize, and not very stable.

Silver butyrate is yellowish-white, crystalline, and almost insoluble in water. It contains 55.38 per cent. of silver.

§ 7. VALERIC ACID, $C^5H^{10}O^2$.—Valeric acid exists in solid human excrements; it is also found in the urine in typhus fever, and in cases of acute atrophy of the liver, this being explained by the fact that the decomposition of leucine (see § 60) yields ammonium valerate abundantly.

It is a colorless, oily liquid, having an unpleasant, penetrating odor, and a burning taste. It is soluble in all proportions of alcohol and ether, but requires 30 parts of water for its solution.

Its alkaline salts are soluble in water, and crystallize with difficulty; they all possess the odor and taste of valeric acid.

Silver valerate contains 51.67 per cent. of metallic silver.

§ 8. CAPROIC ACID, $C^6H^{12}O^2$, exists in the excrements, and probably in the perspiration, as do also CAPRYLIC ACID $C^8H^{16}O^2$, and CAPRIC ACID $C^{10}H^{20}O^2$. The first two are liquid at ordinary temperatures, the latter is solid. All are difficultly soluble in water, easily soluble in alcohol, and have unpleasant odors, recalling that of the perspiration.

DETECTION AND SEPARATION OF THE FATTY ACIDS.

§ 9. The separation of the preceding acids is usually difficult in animal chemistry, because of the comparatively small amount of material which can be operated on. If the acids are to be sought in urine or perspiration, the liquid is distilled, almost to dryness, with dilute sulphuric acid, and the fatty acids are sought in the distillate. If the liquid to be examined be serous, it is first freed from albumen and the coloring matter of the blood, and then distilled with dilute sulphuric acid as before.

The distillate in either case is saturated with potassium carbonate, evaporated to dryness, and again distilled

with sulphuric acid. The liquid in the receiver will separate into two layers. The upper, oily layer contains the slightly soluble acids, from valeric to capric included. It is decanted, neutralized with baryta-water, and set aside to crystallize. The barium salts separate in the following order: barium caprate in fine, microscopic prisms, containing 28.6 per cent. of barium; barium caprylate in granules, containing 32.38 per cent. of barium; barium caproate in prismatic crystals grouped in hemispherical masses, and containing 37.33 per cent. of barium; barium valerate in large plates, resembling cholesterin, and containing 40.41 per cent. of barium. These salts must be purified by repeated crystallizations, and their proportion of barium determined in order to recognize the acid.

The aqueous layer, which may contain the lower members of the series, from butyric to formic included, is saturated with sodium carbonate, and evaporated to a syrupy consistence. If acetic acid be present, sodium acetate crystallizes out; the mother liquor is then again distilled with sulphuric acid, and the oily layer of the distillate is separated and again distilled alone; the portion which passes between 120 and 140° is collected apart, neutralized with ammonia, precipitated by silver nitrate, and boiled until the precipitate is entirely dissolved. Any formic acid present will be decomposed, producing a black deposit of silver; this is separated by filtration, and, on cooling, the solution will deposit crystals of silver propionate. That portion of the liquid which passes between 141 and 160° may contain butyric acid; it is collected apart, saturated with baryta-water, and allowed to crystallize. Barium butyrate, purified by recrystallization, contains 48.41 per cent. of barium.

Lactic Acid.



§ 10. There are two lactic acids found in the economy, both having the same composition and probably the same molecular structure. The one is formed by the fermentation of lactose and glucose in presence of alkalies, and

is known as lactic acid of fermentation; it is found in variable and uncertain quantities in the contents of the stomach and in the intestinal canal, resulting probably from the fermentation of certain articles of food. The other acid, called paralactic or sarcolactic, exists normally in the muscular juices, possibly in the gastric juice, and has been detected as a pathological constituent of the urine after poisoning by phosphorus. Although much labor has been devoted to the study of these acids, the question of their isomerism is by no means certainly decided; it seems to be a case of physical isomerism, and the acids differ more in their salt-forming powers than in their chemical properties. Paralactic acid is optically active, rotating the plane of polarized light towards the right.

A third isomeride, hydracrylic acid, may perhaps exist in the muscular juices, together with paralactic acid, but the matter is involved in doubt.

§ 11. Lactic acid of fermentation may be prepared by allowing a mixture of 3 kilos of glucose dissolved in 13 litres of water, 4 kilos of sour milk, 100 grammes of old cheese, and 1.5 kilo of chalk to ferment for a week at a temperature of about 35° . The calcium lactate formed is recrystallized, decomposed by sulphuric acid, and the filtered liquid boiled, and saturated with zinc hydrocarbonate. After the crystallization of the zinc lactate, it is dissolved, decomposed by hydrogen sulphide, and the filtered liquid is concentrated on a water-bath. The acid is a syrupy, colorless liquid, which is altered and finally decomposed by heat.

§ 12. Paralactic acid may be prepared from extract of meat: this is extracted with weak alcohol, the alcohol distilled off, and the liquid residue acidulated with sulphuric acid, and agitated with ether. The ether, being separated by distillation, leaves impure paralactic acid which is converted into zinc salt, and the latter is purified by repeated crystallization, and decomposed by hydrogen sulphide.

Paralactic acid much resembles its isomeride, already considered. By oxidation, both acids yield acetic and formic acids.

§ 13. *Zinc lactate*, $\text{Zn}(\text{C}^3\text{H}^5\text{O}^3)^2 + 3\text{H}^2\text{O}$, contains

18.18 per cent. of water of crystallization, and is but slightly soluble in water; it separates from boiling aqueous solutions in crystalline needles or prisms, often grouped in tufts. It is almost entirely insoluble in alcohol.

Zinc paralactate, $\text{Zn}(\text{C}^3\text{H}^5\text{O}^3)^2 + 2\text{H}^2\text{O}$, contains 12.90 per cent. of water of crystallization, and is much more soluble in water than the corresponding salt of ordinary lactic acid. It also dissolves freely in alcohol.

The differences in the solubilities and percentage of water of crystallization of these two salts is sufficient to enable the distinction of lactic from paralactic acid.

§ 14. If it be desired to test for lactic acid in urine, the latter is evaporated to dryness on a water-bath, and the residue is extracted with an alcoholic solution of oxalic acid; the filtered solution is then digested with lead oxide, again filtered, and the filtrate decomposed by hydrogen sulphide. After evaporation, the liquid separated from the precipitated lead sulphide, leaves any lactic acid present, and the latter is converted into zinc salt by boiling with zinc oxide. The zinc lactate or paralactate is recognized by the characters mentioned in the preceding section.

Oxalic Acid.



§ 15. Free oxalic acid does not exist in the animal economy; it is found only as calcium oxalate, principally in urinary sediments and calculi. It is very frequently found in human urine, particularly after the ingestion of certain vegetable aliments, and after the internal use of alkaline acid-carbonates.

It crystallizes in large, transparent, oblique-rhombic prisms, containing two molecules of water of crystallization. It melts in its water of crystallization at 98° , and becomes anhydrous at 100° ; at 132° it begins to decompose, and at about 155° breaks up into water, carbon monoxide, carbon dioxide, and formic acid. It is soluble in about 16 parts of water, and dissolves also in alcohol.

All of the oxalates are decomposed by a red heat,

disengaging carbon monoxide and carbon dioxide. The alkaline and earthy oxalates are then converted into carbonates, while the other metallic oxalates leave a residue of oxide or of metal. With the exception of the alkaline oxalates, these salts are only very slightly soluble in water, or entirely insoluble.

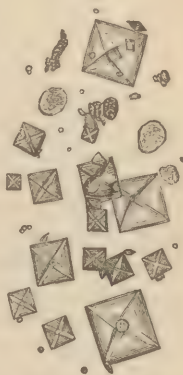
Lime-water and soluble salts of calcium produce, in solutions of oxalic acid or alkaline oxalates, a white precipitate of calcium oxalate, which dissolves in hydrochloric and nitric acids, but is insoluble in acetic acid. It is also very soluble in sodium acid phosphate, and if this solution be neutralized, drop by drop, with a dilute solution of sodium hydrate, the calcium oxalate separates in regular, characteristic crystals.

When dry oxalic acid or an oxalate is heated with concentrated sulphuric acid, carbon monoxide and carbon dioxide are disengaged, and if the experiment be made with an appreciable quantity of the substance in a test-tube, the gas may be lighted at the mouth of the tube.

§ 16. *Calcium oxalate*.—It is of importance to be able to recognize this salt, as it frequently occurs as a urinary sediment and a constituent of vesical calculi. Artificially prepared calcium oxalate occurs as a white powder, devoid of crystalline structure; as has already been mentioned, it may be obtained crystallized by slowly precipitating it from its solution in sodium acid phosphate. It then presents the same appearance under the microscope as the crystals which are found in the urine. It forms characteristic, brilliant, and transparent, regular octahedra, somewhat resembling the reverse side of an envelop (Fig. 2). It is entirely insoluble in both hot and cold water, and in acetic acid, but dissolves in the strong mineral acids. When heated, it is converted into calcium carbonate, without carbonization.

Calcium oxalate is identified by its crystalline form.

Fig. 2.



Calcium oxalate.

It may possibly be held in solution by the sodium acid phosphate in very acid urine, but in this case it is precipitated in crystals when the urine is gradually neutralized by sodium hydrate.

It sometimes occurs in curious, dumb-bell shaped crystals, such as are represented in figure 3. When dry,

Fig. 3.



Dumb-bell crystals of calcium oxalate.

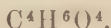
Fig. 4.



Dry calcium oxalate.

the ordinary octahedral crystals appear opaque, with the exception of the centre which presents a brilliant square (Fig. 4); this appearance is caused by the high refractive power of the crystals, and they may again be rendered transparent by moistening them with water.

Succinic Acid.



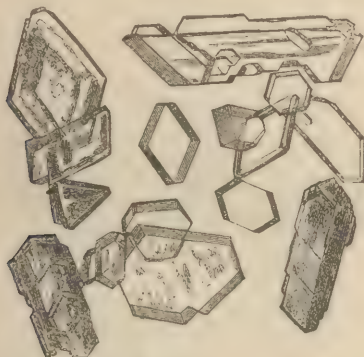
§ 17. Succinic acid has been found in human urine, and in the perspiration and saliva, after the ingestion of benzoic acid. It is also found in the urine after the ingestion of vegetables containing asparagin, and is present in the glandular juices, in the liquid contained in hydatid cysts, and in hydrocele.

It crystallizes from its aqueous solution in brilliant rhomboidal plates, derived from a right-rhombic prism; the angles of these plates are sometimes wanting, giving them the appearance of hexagonal tables (Fig. 5). It is colorless, odorless, and has a feeble, acid taste. It is very soluble in water, soluble in boiling alcohol, very slightly soluble in cold absolute alcohol and in ether.

It melts at 180° , and boils at 235° , but if heated rapidly it sublimes, giving off irritating vapors. It is quite

stable, and is not attacked by nitric acid; when heated with potassium hydrate, it yields potassium oxalate. The alkaline succinates are freely soluble in water, but insoluble in absolute alcohol.

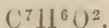
Fig. 5.



Succinic acid.

According to Meissner, succinic acid may be detected in the urine as follows: Baryta-water is added to the urine as long as a precipitate forms; the mixture is filtered; the filtrate is exactly neutralized with sulphuric acid, and the barium sulphate formed is separated by filtration. The new filtrate is evaporated, so that the urates and urea may crystallize out, and the clear liquid is then diluted with absolute alcohol until its bulk is about equal to that of the urine employed. The deposit formed is exhausted with a little water, and the aqueous solution of sodium succinate so obtained is allowed to crystallize. When the crystals are treated with sulphuric acid, succinic acid is set free, and may be recognized by its crystalline form, its fusing point, and its stability when heated.

Benzoic Acid.



§ 18. Benzoic acid is sometimes present in urine, being a product of the decomposition of hippuric acid, or

derived from the ingestion of a large dose of the acid. By sublimation, it crystallizes in long flexible needles; but when deposited from its aqueous solution, it crystallizes in rectangular tables, easily recognized under the microscope (Fig. 6).

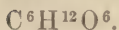
Fig. 6.



Benzoic acid.

For its detection in the urine or other animal fluid, the latter is rendered alkaline by sodium carbonate, and evaporated to dryness on a water-bath. The residue is exhausted with alcohol, and the alkaline solution treated with a little hydrochloric acid. Should no crystals of benzoic acid separate, the matter is exhausted with ether, and the solution is either left to spontaneous evaporation, or mixed with water, which will cause the benzoic acid to separate in the crystallized state. Better crystals are obtained by allowing the ethereal extract to evaporate until it becomes thick and oily, and then treating it with water. Once obtained, no difficulty will be experienced in identifying the acid.

If a little benzoic acid be mixed with a small quantity of nitric acid, and the mixture be boiled in a porcelain capsule, as the residue becomes concentrated, a strong odor of nitrobenzol, resembling that of oil of bitter almonds, is developed.

Glucose.

§ 19. Glucose exists normally in the blood, especially in that of the hepatic vein, in the lymph, the fluids of the liver, and in the chyle and contents of the small intestine after the ingestion of starchy or saccharine substances. Its normal occurrence in urine is doubtful, but it is often found in large quantities in that liquid in certain pathological conditions, especially in *diabetes mellitus*.

Pure glucose is a white solid, occurring in small crystalline, cauliflower-like masses containing one molecule of water of crystallization. It melts when heated, loses its water of crystallization at 100° , and again solidifies. Anhydrous, it melts at 144° . It is soluble in a little more than its weight of cold water, and is much less soluble in alcohol. Its solutions rotate the plane of polarized light towards the right.

§ 20. DETECTION.—Glucose is detected by the facility with which it reduces certain metallic salts, such as cupric salts, bismuth salts, etc., in alkaline solutions. The liquid to be tested should contain no albuminoid matter; if such be present, the liquid is treated with a few drops of acetic acid, boiled and filtered; or, if much albumen be present, as in the case of blood, the liquid is mixed with three or four times its volume of strong alcohol, allowed to stand for some time, heated and filtered; the filtrate is evaporated to dryness on a water-bath, and the residue is exhausted with water. Or, again, the liquid may be rendered acid by acetic acid, saturated with crystallized sodium sulphate, heated to boiling, and filtered: by this method albumen and glucose may be successively sought for in the same liquid.

§ 21. If a solution of glucose be made strongly alkaline with potassium or sodium hydrate and heated to boiling, the liquid assumes a yellow, brown, or black color, according to the proportion of glucose present (Moore). Traces of glucose give a yellow tint.

§ 22. When a solution of glucose is mixed with potassium hydrate, and then agitated with a few drops of a

dilute solution of cupric sulphate, no precipitate of cupric hydrate is formed, but the liquid acquires a blue color. If now it be heated to boiling, a yellow or red precipitate of cuprous oxide is thrown down (Trommer). An excess of cupric sulphate must be avoided, lest a black precipitate of the cupric oxide in excess mask the color of the cuprous oxide. An excess of alkali is not detrimental, for if more glucose be present than is required to reduce the cupric oxide, the liquid above the precipitate becomes yellow or reddish-brown, as indicated in the preceding paragraph.

§ 23. Much better results may be obtained by the use of *Fehling's solution*, which is prepared as follows: 34.64 grammes of pure, crystallized cupric sulphate are dissolved in about 200 c. c. of distilled water; 173 grammes of sodium and potassium tartrate are dissolved in a solution of about 80 grammes of sodium hydrate in 600 c. c. of distilled water; the solution so obtained is poured into the copper solution and the mixture agitated, and diluted with distilled water to exactly 1 litre. This liquid has a dark-blue color, and must be preserved in well-stoppered bottles in a cool, dark place. It is used also for quantitative estimations, and 1 c. c. of it is exactly precipitated by 5 milligrammes of glucose.

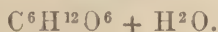
In using this solution, about 5 c. c. are poured into a test-tube and heated to boiling. The liquid will remain clear if it be good. The suspected solution is then poured in, drop by drop, and, if glucose be present, the blue color will change to green, and almost immediately to yellow and red, cuprous oxide being precipitated. If only traces of glucose be present, it may be necessary to add several c. c. of the suspected solution, and to boil the mixture during one or two minutes.

§ 24. Glucose reduces alkaline solutions of bismuth oxide at the temperature of ebullition, precipitating metallic bismuth (Böttger). The reagent may be prepared by moistening a mixture of 5 grammes of bismuth basic nitrate and 5 grammes of pulverized tartaric acid with about 30 c. c. of distilled water, and carefully adding, with continual agitation, strong solution of sodium hydrate until the whole is dissolved. The reagent does not keep

well, and must be preserved in well-stoppered bottles. If some of this solution be boiled with a liquid containing glucose, a brown or black pulverulent precipitate of metallic bismuth will be formed. It is essential that the suspected solution be free from albumen, as the sulphur in the latter body would occasion a black precipitate of bismuth sulphide.

Under the influence of yeast, glucose rapidly undergoes fermentation, yielding alcohol and carbon dioxide.

Inosite.



§ 25. Inosite is present in small quantity in the muscular tissue of the heart, in the liver, lungs, pancreas, spleen, and kidneys. Pathologically, it is sometimes found in the urine of individuals suffering from diabetes or Bright's disease, and in the muscles of habitual drunkards.

It may be extracted from the juices obtained by expression from the muscles, glandular tissues, lungs, etc. The albumen is coagulated by heat, the phosphates are precipitated by addition of baryta water, and the mixture is filtered; the filtrate is concentrated, and, after the creatine has crystallized out and been separated, the aqueous liquid is mixed with several times its volume of hot, strong alcohol. If the precipitate formed be abundant and adhere to the vessel, the alcohol is decanted; but, if it be not viscous, the mixture is filtered boiling, and the filtrate allowed to stand 24 hours. The crystals of inosite that separate are collected on a filter and washed with a little cold alcohol. They are then redissolved in a little boiling water, and the hot solution is mixed with several times its volume of strong alcohol and allowed to cool slowly; the inosite then separates in delicate crystals. If in either of these operations the addition of alcohol occasion no precipitate, the solution is agitated with ether until it becomes turbid, and then allowed to stand (Boedecker).

§ 26. Inosite may also be separated by precipitating the phosphates, albumen, etc., by a solution of neutral

lead acetate, then filtering, and adding basic lead acetate, which precipitates inosite. The precipitate is washed, suspended in water, and decomposed by hydrogen sulphide; the filtered solution is then concentrated and precipitated by hot, strong alcohol, as directed in the preceding paragraph. This method is applicable to urine suspected to contain inosite.

§ 27. Inosite crystallizes from its aqueous solutions in large oblique-rhombic prisms or tables, containing one molecule of water, which they lose in dry air. The small crystals are usually grouped in tufts. It is quite soluble in water, and the solution has a sweet taste. It is optically inactive, will not reduce Fehling's solution, and will not ferment under the influence of yeast.

§ 28. If a small quantity of inosite be moistened with nitric acid, on a piece of platinum foil, and evaporated nearly to dryness, the residue will acquire a bright rose-red color when treated with a drop of ammonia and a little calcium chloride, and again carefully evaporated to dryness. This test is very delicate, but succeeds only with pure inosite (Scherer). These reactions serve to distinguish between inosite and glucose.

Lactose.



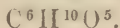
§ 29. Lactose exists in the milk of all herbivorous mammals, in woman's milk, and in that of the dog. It probably exists also in the milk of all mammals.

It is prepared by evaporation of the whey which remains after the manufacture of cheese, the crystals which separate being subjected to several recrystallizations. It occurs in colorless right-rhombic prisms, terminated by octahedral pyramids, and containing one molecule of water, which they lose at about 140° . They are hard, and grate when crushed between the teeth. Lactose requires about 6 parts of cold or 2 parts of boiling water for its solution, and is insoluble in absolute alcohol. Its solutions are dextrogyrate.

§ 30. Under the influence of dilute acids, lactose is converted into glucose, and an isomeric sugar which is known as galactose. The solution will then undergo the alcoholic fermentation. In presence of casein, alkaline solutions of lactose yield various fermentation products, principally lactic acid, which is finally converted into butyric acid.

Lactose reduces Fehling's solution, even in the cold, but less readily than glucose, from which it may be distinguished by its crystalline form and its much more difficult and imperfect fermentability under the influence of yeast.

Glycogen.



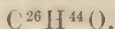
§ 31. This compound, which is isomeric with the starches, was discovered by Bernard in the tissue of the liver, and afterwards in the placenta; it exists also in nearly all the organs during foetal life. It may be obtained by macerating finely divided liver, previously deprived of its blood, with water, acidifying the opalescent decoction with acetic acid, heating to boiling, and separating the coagulum by filtration. The filtrate is mixed with twice its volume of strong alcohol, and, after standing for several hours, is again filtered. The precipitate on the filter is washed with alcohol, dissolved in water, and again treated with a little acetic acid, boiled and filtered, and the clear liquid precipitated by alcohol. The precipitate, consisting of pure glycogen, is collected on a filter and washed with a little ether.

§ 32. So obtained, glycogen is a snow-white, amorphous powder. When dried at low temperatures, it contains one molecule of water, but becomes anhydrous at 100° . It swells in cold water, and quickly dissolves by the aid of heat, forming an opalescent liquid which becomes clear on the addition of potassium hydrate. It is insoluble in alcohol and ether; its aqueous solution is dextrogyrate.

Boiling with dilute acids converts it into glucose, and

the same change is brought about by the action of saliva, pancreatic juice, diastase, and other ferments. Iodine, employed in small quantity, colors it brown, dark red, or even violet; but the tint can readily be distinguished from the blue color produced by that reagent with starch. Glycogen may also be distinguished from the latter body by its amorphous appearance under the microscope.

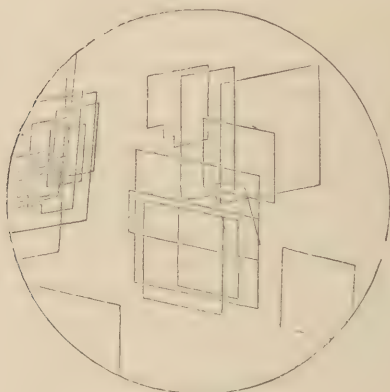
Cholesterin.



§ 33. This compound is widely distributed in the human body. It exists in the brain and nerve tissues, the serum of blood, yolk of egg, bile, and excrements. It is the principal constituent of most biliary calculi, and also occurs pathologically in pus and in dropsical effusions, especially in ovarian dropsy and in hydrocele.

Cholesterin may be easily extracted in a pure state from biliary calculi. These are crushed, exhausted with boiling water to remove pigments and mineral salts, and

Fig. 7.



Cholesterin.

the dry residue is boiled with alcohol. The alcoholic solution is filtered while hot, and the cholesterin crystallizes out on cooling. It may be purified by recrystal-

lization in alcohol or ether. It is readily soluble in ether and in hot alcohol, from which it separates on cooling in thin, brilliant, transparent, and colorless rhombic plates, of which the edges are generally broken and somewhat irregular (Fig. 7). It is also soluble in benzol and in chloroform, but entirely insoluble in water.

It is tasteless and odorless, somewhat greasy to the touch. It melts at 145° , and may be sublimed, out of contact with air, at 360° . Its solutions are neutral, and deviate the plane of polarized light towards the left.

If cholesterol be moistened with concentrated sulphuric acid, triturated, and then agitated with chloroform, a blood-red or violet solution is obtained, which gradually becomes colorless on contact with the air, passing successively through the tints of violet, blue, and green.

The appearance of thin transparent plates in an animal liquid, under microscopic examination, would indicate the probable presence of cholesterol; the latter may be then extracted by treatment with ether, but its separation from fatty and soapy matters is often exceedingly difficult.

STERCORIN AND SEROLIN.

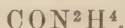
§ 34. When dried serum of blood is treated with a large quantity of boiling alcohol, and the liquid filtered, white pearly scales are deposited on cooling. This substance was named serolin by Boudet. Flint subsequently obtained it in considerable quantity from the excrements, and named it stercorin. It melts at 36° , dissolves readily in ether, but not so well in cold alcohol, and is neutral to litmus-paper. It is colored red by concentrated sulphuric acid, like cholesterol. Hoppe-Seyler regards it as a mixture of cholesterol, lecithine, etc.; derived from the blood.

EXCRETIN.

§ 35. This compound was obtained by Marcet from human excrements. The latter are exhausted with boiling alcohol, and the cold filtered extract is precipitated by calcium hydrate; the precipitate is dried and treated

with hot alcohol and ether; after a few days, these solutions deposit excretin in prismatic crystals, which are insoluble in water, almost insoluble in cold alcohol, but readily soluble in hot alcohol and in ether. It melts at $92-96^{\circ}$, and is unattacked by boiling alkaline and acid solutions, excepting nitric acid. It contains sulphur.

Urea.



§ 36. Urea is the principal solid constituent of human urine, the greater part of the nitrogen produced by the waste of the tissues being eliminated in this compound. It exists also in the blood, in the exudations, and in very small quantity in the aqueous humor and the lymph. When for any reason its elimination by the kidneys is arrested or interfered with, it accumulates in the liquids of the body, and may be detected in the perspiration and the saliva.

Pure urea crystallizes in long, four-sided rhombic prisms, terminated by obtuse pyramids of which only one or two faces are developed. They are white, silky, and striated. They are anhydrous, and may be heated to 120° without decomposition; above that temperature they melt, disengaging ammonia.

Urea is soluble in about its own weight of cold water, and in the same quantity of boiling alcohol; almost insoluble in ether. Its aqueous solution is neutral, but slowly decomposes after a time, more rapidly by the action of heat.

§ 37. The strong mineral acids and the alkaline hydrates decompose urea, with the formation of carbon dioxide and ammonia.

By the action of nitrous acid, it is converted into water, nitrogen, and carbon dioxide.

§ 38. Chlorine and the alkaline hypochlorites decompose it, with formation of hydrochloric acid, water, and carbon dioxide, and liberation of nitrogen. Bromine and the alkaline hypobromites act in a similar manner.

§ 39. If a solution of urea be mixed with a solution of mercuric nitrate, a precipitate is formed having a variable

composition, being a mixture of compounds containing two molecules of urea nitrate combined with two, three, and four molecules of mercuric oxide. If a dilute solution of mercuric nitrate be slowly added to a dilute solution of urea, and the mixture be neutralized from time to time with sodium carbonate, a moment will arrive when the addition of the latter compound will communicate to the precipitate a yellow color, indicating the presence of free mercuric nitrate. The whole of the urea is then precipitated, and the precipitate contains two molecules of urea nitrate and four molecules of mercuric oxide (Liebig).

These reactions are employed for the estimation of urea.

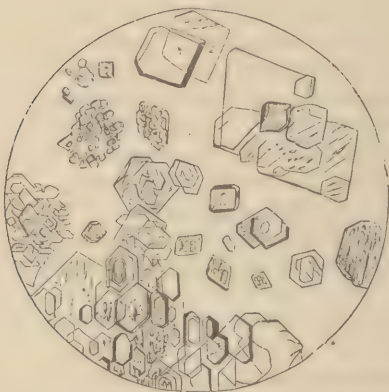
§ 40. Urea may be extracted from urine by precipitating the phosphates and sulphates by a mixture of one volume of barium hydrate and two volumes of barium nitrate, both in saturated solution, filtering, evaporating the filtrate to dryness on a water-bath, and exhausting the residue with hot absolute alcohol. The alcoholic solution is allowed to crystallize, and if the product obtained be colored, it is redissolved in alcohol, filtered through animal charcoal, and again crystallized.

UREA NITRATE.



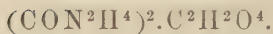
§ 41. Urea combines with a number of acids, forming well-marked crystalline compounds. The nitrate, which is characteristic, may be obtained by adding moderately strong nitric acid to a concentrated solution of urea, and cooling the mixture. The compound separates in brilliant white scales or plates, or in prismatic crystals. The forms of these crystals may be well studied by bringing in contact a drop of nitric acid and a small crystal of urea upon a glass slide on the stage of the microscope (Fig. 8).

Fig. 8.



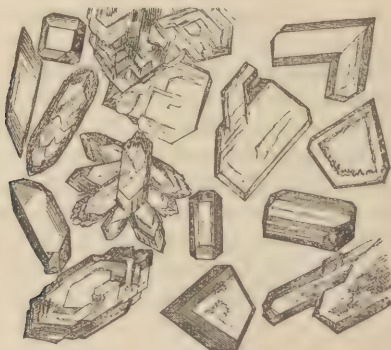
Urea nitrate.

UREA OXALATE.



§ 42. This compound is obtained by mixing concentrated solutions of urea and oxalic acid. The crystals are sometimes very similar to those of the nitrate, but

Fig. 9.



Urea oxalate.

generally present such great variations that they are by no means as characteristic and certain as those of the latter salt (Fig. 9).

DETECTION OF UREA.

§ 43. Urea is recognized by the crystalline forms of its nitrate and oxalate. If the liquid to be examined be free from albumen, it is evaporated to dryness, exhausted with absolute alcohol, the solution evaporated, and the residue treated with pure nitric or oxalic acid on a microscope slide. If the liquid be serous, it is mixed with three or four times its volume of strong alcohol, which precipitates the albumen and a great part of the salts; it is then filtered and evaporated to dryness, and the residue extracted with absolute alcohol as before. Very small quantities of urea may be thus detected.

Care must be taken not to confound the crystals of urea nitrate with those of an alkaline nitrate, which they resemble, and which might be obtained in the examination for urea of blood or other serous liquid. The distinction is easily made by heating the crystals on a piece of platinum foil or on the microscope slide. An alkaline nitrate will then fuse and leave a fixed white residue, while urea nitrate will completely volatilize, or leave a mere trace of residue in case the salt be not perfectly pure.

Crystals of urea nitrate may be easily obtained from urine by evaporating the latter to about one-fifth its volume, filtering, and adding about an equal quantity of somewhat dilute nitric acid (specific gravity 1.25). The liquid often solidifies to a crystalline mass. The crystals may be drained, redissolved in the smallest possible quantity of boiling water, and the solution decolorized by hot filtration through animal charcoal. On cooling, colorless crystals of urea nitrate will be deposited.

Hippuric Acid.

§ 44. Hippuric acid exists in the urine of the horse and many other herbivorous animals, and in small quantity in human urine; it is generally found as an alkaline salt or in combination with calcium. The proportion

existing in human urine is much increased by an exclusively vegetable diet.

Benzoic acid is converted into hippuric acid in the system, and the latter acid may be readily obtained from human urine by administering twenty-five or thirty grammes of benzoic acid in the evening, and treating the urine passed during the night and the following morning. This is mixed with milk of lime, and boiled for a few minutes; the liquid is filtered hot, and the filtrate rapidly evaporated to one-sixth or one-eighth of its volume and saturated with hydrochloric acid. The hippuric acid which is precipitated is collected on a filter, again boiled with milk of lime, filtered, and the filtrate precipitated by hydrochloric acid. Should the acid still remain colored, it may be completely decolorized by dissolving it in boiling water, adding animal charcoal, and filtering while boiling. Putrid urine will not yield hippuric acid.

§ 45. Hippuric acid crystallizes in long colorless prisms (Fig. 10), slightly soluble in cold water and in

Fig. 10.



Hippuric acid.

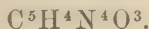
ether; very soluble in boiling water and in alcohol. It is odorless, but has a somewhat bitter taste. Its solutions strongly redden blue litmus.

When hippuric acid is heated in a tube, it melts and

yields a sublimate of benzoic acid; at the same time a small quantity of benzonitrile, an oily liquid having an unpleasant odor, distils and condenses in red, oily drops on the sides of the tube.

If hippuric acid be boiled with concentrated nitric acid, and the mixture be evaporated to dryness and heated in a small tube, the characteristic odor of nitrobenzol will be perceptible.

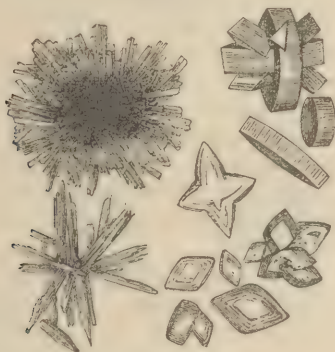
Uric Acid.



§ 46. Uric acid is constantly present in normal human urine, and in that of carnivorous animals; it is also found in the urine of herbivores, but in much smaller quantity. It constitutes the entire mass of certain vesical calculi, and, either free or in combination, the gouty concretions sometimes formed in the joints. It exists in small quantity in human blood, and in large quantity in the excrements of birds and serpents, and in guano. The quantity excreted in the urine of a healthy adult varies from one to three or four decigrammes per day.

The process indicated for its quantitative estimation (§ 151) serves for its preparation.

Fig. 11.



Uric acid.

§ 47. Pure uric acid is a light, white powder, composed of microscopic crystals belonging to the right-

rhombic system (Fig. 11). It is tasteless and odorless, and only slightly soluble in water. 1 part of uric acid requires from 11,000 to 15,000 parts of cold water, or from 1800 to 1900 parts of boiling water, to effect its solution. It is entirely insoluble in alcohol and ether. It is insoluble in hydrochloric acid, but dissolves readily in concentrated sulphuric acid from which it is again precipitated by the addition of water. Its aqueous solutions are almost without action on litmus paper, even when saturated at the temperature of ebullition.

§ 48. Solutions of the alkalies dissolve uric acid readily, forming neutral urates; uric acid is dibasic, and the acid urates are much less soluble than the neutral salts.

Uric acid is not volatile; when heated in the air, it burns, leaving no residue. When heated in a tube, it blackens, disengages urea, hydrocyanic acid, oily pyrogenous products, and some cyanuric acid which condenses in crystals on the sides of the tube. The residue is a nitrogenized carbon.

§ 49. Uric acid dissolves in moderately strong nitric acid, with decomposition and the formation of a yellow solution; nitrogen and carbon dioxide are disengaged, and the residue consists of alloxane* and urea. If this solution be cautiously evaporated to dryness, the residue assumes a red color, and if it be moistened with very dilute ammonia, a rich purple color will be developed. The names *murexide* and *ammonium purpurate* have been given to the purple compound so formed. If the ammonia be replaced by a solution of potassium hydrate, a blue color will be obtained.

Caffeine gives a similar color with nitric acid, and the residue becomes purple when moistened with ammonia, but potassium hydrate produces no effect.

Solutions of the alkaline urates reduce Fehling's solution, precipitating Cu^2O .

§ 50. Uric acid is detected by the murexide test, and by its crystalline form as shown by the aid of a microscope.

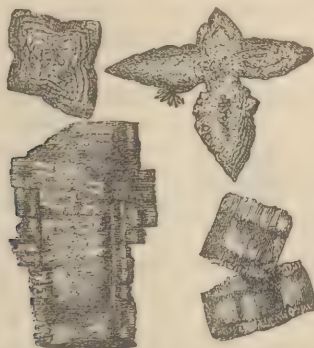
* $\text{C}_4\text{H}_2\text{N}_2\text{O}_4$. This body was found by Liebig in the mucus of an intestinal catarrh. This is probably the only certain instance of its detection in the body.

The murexide test is conveniently made in a watch-glass or small porcelain capsule, in which the substance suspected to contain uric acid is placed, and, if liquid, evaporated to dryness. It is then moistened with nitric acid and gently heated over a flame or on a water-bath. If much uric acid be present, a considerable effervescence takes place: this terminated, the residue is cautiously evaporated to dryness, the capsule being moved so as to spread the liquid over its surface. The residue, which was at first pale-yellow, gradually becomes bright-red as the excess of acid is expelled. The red color is a proof of the presence of uric acid, but the residue should be moistened with a drop of ammonia, or better, a glass rod bearing a drop of ammonia may be held near the residue until the fumes produce the required effect. Under these circumstances, the presence of uric acid is indicated by a rich purple color.

EXTRACTION FROM SMALL QUANTITIES OF LIQUID.

§ 51. If it be desired to extract the uric acid from a small quantity of a liquid, such as urine, the latter, of

Fig. 12.

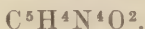


Uric acid precipitated by hydrochloric acid.

which at least 5 or 6 c.c. should be used, is placed in a large watch-glass, and five or six drops of concentrated hydrochloric acid are added. A linen thread is introduced into the mixture, and the glass is covered and set

aside for twenty-four hours in a cool place. The uric acid slowly crystallizes on the thread, and may be examined by the aid of a microscope. When so precipitated by hydrochloric acid, the crystals usually present the forms shown in Fig. 12.

Xanthine.



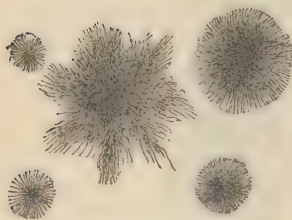
§ 52. Xanthine is found in certain rare urinary calculi, and exists normally in the urine, liver, spleen, brain, and muscular tissues, only, however, in very small quantity. (See § 196.)

It is a hard, pale-yellow substance, which assumes a wax-like appearance by friction. It is scarcely soluble in cold water, but dissolves in boiling water; almost insoluble in alcohol and ether. After the evaporation of its aqueous solution it is deposited in a leaf-like film. When heated, it decomposes without melting.

It dissolves in nitric acid, by the aid of heat, without evolving gas, and the solution leaves a yellow residue after evaporation, which is colored reddish-yellow by potassium hydrate, and becomes reddish-violet when heated.

Xanthine dissolves also in potassium hydrate, and in ammonia, and on the evaporation of its ammoniacal solution is deposited in groups of brilliant laminæ.

The detection of xanthine in urinary calculi is not difficult, since xanthic calculi are composed almost entirely of that body. The calculus is dissolved in potassium hydrate, the xanthine precipitated by hydrochloric acid,



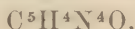
Xanthine and silver nitrate.

and characterized by its solubility in ammonia, its reaction with nitric acid and potassium hydrate, and by the fact that its nitric acid solution yields with silver nitrate a flocculent precipitate, which dissolves on boiling, and

is again deposited in groups of microscopic needles on cooling (Fig. 13).

For the extraction of xanthine from urine, 50 or 100 litres of that liquid are necessary.

Hypoxanthine (sarcine).



§ 53. Hypoxanthine exists in small quantity in the muscular tissues, spleen, liver, and in the marrow of the bones. Larger quantities are found in the liver when that organ is in the diseased condition known as acute yellow atrophy.

It is only slightly soluble in cold water, more soluble in boiling water; not very soluble in alcohol. It forms microscopic, colorless needles, and is deposited on the cooling of its boiling aqueous solution, in white flakes. It much resembles xanthine, from which it can only be separated with difficulty. (See § 196.)

It is decomposed when heated above 150° . It dissolves easily and without decomposition in ammonia and in alkaline or slightly acid solutions. The residue left by the evaporation of its nitric acid solution is not affected by potassium hydrate, unless heat be applied.

Fig. 14.



Hypoxanthine and silver nitrate.

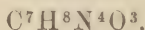
If ammoniacal silver nitrate be added to an ammoniacal solution of hypoxanthine, a double compound of silver and hypoxanthine nitrates separates as a colorless, gelatinous precipitate; this is insoluble in water and in ammonia, but dissolves somewhat in boiling nitric acid. If

this solution be allowed to cool very slowly and evaporate in a watch-glass, large prismatic crystals are obtained, often grouped in stars (Fig. 14). The compound of xanthine and silver nitrate is much more soluble in nitric acid, and separates from its solutions very slowly, sometimes only after the lapse of several days.

GUANINE.

§ 54. By the action of reducing agents, such as ferrous sulphate, hypoxanthine is converted into guanine, $C^5H^5N^5O$, a body which was first obtained from guano, but has since been found in the pancreas and in the liver. It is a yellowish-white solid, tasteless, and odorless, insoluble in water, alcohol, and ether, but soluble in acids and in solutions of the alkaline hydrates.

Carnine.



§ 55. This compound was discovered by Weidel in commercial extract of meat. He prepared it by dissolving the extract in five or six times its weight of warm water, and adding saturated baryta-water to the solution as long as a filtrate was formed, at the same time avoiding an excess of baryta. When the clear liquid obtained by filtering this mixture is treated with basic lead acetate, a precipitate is formed which contains nearly all of the carnine in a state of combination with the lead. The precipitate is separated by filtration, boiled with water, which then dissolves the compound of lead and carnine, and the mixture is filtered boiling. A current of hydrogen sulphide is passed through the filtrate, and the lead sulphide is removed by filtration. The filtrate is concentrated to a small bulk, and allowed to stand until part of the carnine is deposited in dark-colored crystalline masses. After separating this deposit, the mother-liquor is treated with a solution of silver nitrate, which precipitates silver chloride, and a silver compound of carnine; the latter is only slightly soluble in ammonia; the precipitate is therefore treated with ammonia, which dissolves the silver chloride, and the compound of silver

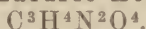
and carnine is washed with water, suspended in boiling water, and decomposed by hydrogen sulphide. The filtered liquid is then concentrated, decolorized with animal charcoal, and allowed to crystallize. Notwithstanding inevitable losses, this process yields about one per cent. of carnine.

Carnine forms small, white, poorly-crystallized masses, which are only slightly soluble in cold water, but dissolve readily in boiling water. It is insoluble in either alcohol or ether. Its aqueous solution, which is neutral, is not precipitated by neutral lead acetate, but yields an abundant precipitate with basic lead acetate, if no neutral acetate be present.

It combines with hydrochloric acid, forming a hydrochloride which crystallizes in brilliant needles and forms a double salt with platinic chloride.

When heated with chlorine water and a trace of nitric acid, it leaves a white residue which assumes a red color when exposed to ammonia vapors. This reaction seems to indicate the formation of hypoxanthine, which acts in the same manner.

Oxaluric Acid.



§ 56. The ammonium salt of this acid is, according to Schunck and Neubauer, normally contained in human urine. The acid itself is a derivative of uric acid, and ammonium oxalurate is formed when parabanic acid* is heated with ammonia.

Large quantities of urine (about 50 litres) are required for the detection of ammonium oxalurate. The fresh urine, having an alkaline reaction, and previously filtered, is filtered through animal charcoal, which absorbs the oxalurate, with much of the other salts. The charcoal is then thoroughly washed with water to remove chlorides and sulphates, and dried at a gentle heat; it is then exhausted with boiling alcohol, the alcoholic liquid is filtered and evaporated to dryness, and the residue extracted with warm water; the brown aqueous

* Oxalyl-ureide, $\text{C}^3\text{H}^2\text{N}^2\text{O}^2$. It is formed by the action of an excess of nitric acid on alloxane or on uric acid.

solution is concentrated to a syrupy consistence, and, after long standing in a cold place, will deposit crystals of ammonium oxalurate. The crystals are washed with absolute alcohol, redissolved in water, and the solution is decolorized by animal charcoal and recrystallized.

Ammonium oxalurate crystallizes in silky white needles, which may be heated to 120° without decomposition. If a tolerably dilute solution of this salt be mixed with calcium chloride and ammonia, no precipitate is formed; but, when the mixture is heated, calcium oxalate is thrown down, and may be recognized by its crystalline form and its chemical properties.

Creatine.



§ 57. If several pounds of flesh be finely divided, digested for some time in cold water, and then thoroughly pressed, a red liquid is obtained which contains a little blood, together with the constituents of the muscular juices. These are principally albumen, creatine, possibly inosite, paralactic acid, butyric acid, and mineral salts, chiefly phosphates and chlorides.

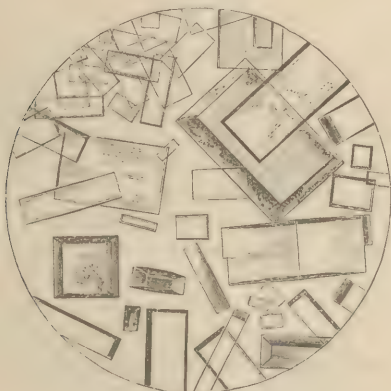
Normal urine contains little or no creatine, that which is sometimes found in it being produced from creatinine (Heintz).

A tolerably large quantity of meat (15 or 20 pounds of beef) must be used for the extraction of creatine, which may be prepared as follows: the flesh is cut into minute fragments, triturated with powdered glass, and then mixed with one and a half times its weight of alcohol and gently heated on a water-bath. The liquid which is extracted by strong pressure is distilled to recover the alcohol, and, after diluting with water and filtration, if necessary, basic lead acetate is added, care being taken not to employ a large excess. The mixture is then filtered, and hydrogen sulphide is passed through the clear filtrate to precipitate the excess of lead, which is separated by another filtration. The solution is then cautiously evaporated to a somewhat syrupy consistence on a water-bath and set aside for crystallization. The

crystals which separate are purified by washing with strong alcohol, dissolving them in water, and recrystallization, after treatment with animal charcoal.

Pure creatine forms brilliant, transparent, and colorless oblique-rhombic prisms (Fig. 15), containing one

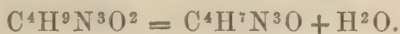
Fig. 15.



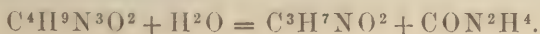
Creatine.

molecule of water, which they lose at 100° , becoming at the same time opaque. It dissolves readily in boiling water, and separates from its saturated solution, on cooling, in fine needles. From more dilute solutions it is slowly deposited in large crystals. It is almost insoluble in strong alcohol, and insoluble in ether. The aqueous solution is neutral, has a somewhat bitter taste, and soon decomposes. With acids, creatine forms crystallizable salts which are soluble in water and readily decomposed.

When treated with strong acids, or by long boiling with water, creatine is decomposed into creatinine and water.



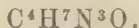
If long boiled with baryta-water it is converted into sarcosine and urea, and, if the action of the reagent be further prolonged, the urea is decomposed into carbon dioxide and ammonia.



When a solution of creatine is boiled with an excess of mercuric oxide, carbon dioxide is disengaged, and metallic mercury is deposited.

Sarcosine, $C^3H^7NO^2$, does not appear to exist in the body.

Creatinine.



§ 58. Creatinine exists normally in human urine, and in that of the dog, horse, and calf. Although it may be extracted from the muscular juices and the blood, it is probably then formed, in operating, from the creatine contained in those liquids. As has already been seen, creatinine may be readily prepared from creatine by boiling with dilute acids, hydrochloric acid answering best. The resulting compound of creatinine and hydrochloric acid is decomposed by an excess of lead hydrate, and the filtered solution is allowed to crystallize.

Creatinine forms colorless, oblique-rhombic prisms (Fig. 16), quite soluble in cold water, more so in boiling

Fig. 16.



Creatinine.

water, and also soluble in boiling alcohol, from which, however, the greater part again separates on cooling.

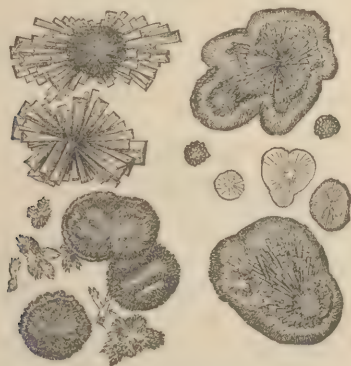
Its solutions have an alkaline reaction, and creatinine forms well-defined salts with the acids.

If silver nitrate be added to an aqueous solution of creatinine, a voluminous, white, crystalline precipitate is formed, consisting of a basic combination of the two compounds.

Mercuric chloride also occasions a white precipitate which soon becomes crystalline.

The most important reaction of creatinine, and one which distinguishes it from creatine, is its behavior with zinc chloride. If a syrupy solution of the latter compound be added to an aqueous solution of creatinine, a white, grainy, crystalline precipitate—a compound of creatinine and zinc chloride—is immediately formed, and the appearance of this precipitate under the microscope is quite characteristic (Fig. 17). It is scarcely soluble

Fig. 17.



Zinc chloride and creatinine.

in cold water, quite insoluble in alcohol. Zinc chloride does not precipitate creatine.

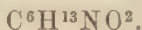
§ 59. DETECTION.—It is sometimes desired to test for creatinine in the urine; for this purpose the latter is neutralized with a little milk of lime, and sufficient calcium chloride is added to precipitate all the phosphoric acid as calcium phosphate. The liquid is then filtered and concentrated on the water-bath. The residue is ex-

hausted with absolute alcohol, and the filtered liquid is allowed to stand several hours, when it is again filtered and mixed with a neutral syrupy solution of zinc chloride. After a time, the double compound of zinc chloride and creatinine separates as a yellow precipitate. This is collected, washed with cold water, then dissolved in boiling water, and the zinc and hydrochloric acid are removed by digestion with lead hydrate. The filtered solution is decolorized by animal charcoal, evaporated to dryness, and the residue, which is a mixture of creatine and creatinine, is treated with boiling alcohol. As this solution cools, the creatine crystallizes out, while the creatinine remains in solution, and may be obtained in crystals by evaporation of the alcohol (Neubauer).

When the creatinine is thus obtained pure, it may be distinguished from creatine by its solubility in cold alcohol, by the alkaline reaction of its concentrated aqueous solution, by its crystalline form, and by its behavior with zinc chloride.

According to Neubauer, 200 or 300 c. c. of urine are sufficient for its detection. It is generally advisable, however, to use a larger quantity.

Leucine.



§ 60. Leucine exists in the glandular organs, and has been found in the lungs, spleen, liver, kidneys, in the thyroid and salivary glands, and especially in the pancreas. It is also met with in the urine of patients suffering from diseases of the liver.

Leucine is a product of the decomposition of albuminoid matters, either by acids, alkalies, or putrefaction. It may be most easily prepared by boiling 2 parts of hornshavings, for about twenty-four hours, with 5 parts of sulphuric acid and 13 parts of water, the latter being replaced as it evaporates. Milk of lime is then added to the mixture, and the precipitated calcium sulphate is separated by filtration. The lime remaining in the filtrate is exactly precipitated by oxalic acid, and the liquid again filtered and evaporated to crystallization.

Leucine and tyrosine are deposited together. They may be separated by rather weak alcohol, in which tyrosine is insoluble; or the mixture may be dissolved in boiling water to which a little ammonia has been added, and the hot solution treated with solution of basic lead acetate and agitated until the precipitate becomes white. The mixture is then filtered, and the filtrate boiled and exactly neutralized with sulphuric acid. Nearly all of the tyrosine is thus precipitated, and, after another filtration, the liquid is freed from lead by a current of hydrogen sulphide and again filtered and boiled. An excess of recently precipitated cupric hydrate is added, and the mixture boiled for some time. The leucine combines with part of the cupric hydrate; the combination, together with the excess of cupric hydrate, is separated by filtration, washed, suspended in water, and decomposed by hydrogen sulphide. A little acetic acid is then added, and the clear liquid obtained by filtration is decolorized by animal charcoal, evaporated to a small bulk, and allowed to crystallize. Nearly pure leucine separates.

§ 61. Leucine crystallizes in small, brilliant, white

Fig. 18.



Leucine.

laminæ (Fig. 18). It is soluble in 27 parts of cold water, and in a much less quantity of boiling water; it

dissolves also in about 1040 parts of cold or in 800 parts of boiling alcohol. It is much more soluble in weak alcohol, insoluble in ether.

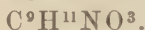
When heated to 170° , it melts and partially sublimes, but above that temperature it decomposes into carbon dioxide and amylamine.

Solutions of leucine are not precipitated by metallic salts, such as those of iron, mercury, silver, or lead, but if a solution of leucine be mixed with neutral lead acetate, the mixture heated to boiling, and ammonia carefully added, a compound of leucine and lead oxide separates in shining plates.

Leucine dissolves in sulphuric, nitric, and hydrochloric acids, forming soluble crystallizable compounds.

If leucine be moistened with nitric acid and cautiously heated on a platinum foil, a colorless residue remains; if this be heated with a drop or two of sodium hydrate solution, it assumes a yellow or brown color, and is finally transformed into an oily compound.

Tyrosine.



§ 62. Tyrosine occurs, together with leucine, in the spleen and pancreas, and in the liver when that organ is diseased, as well as in the urine of persons affected with softening of the liver.

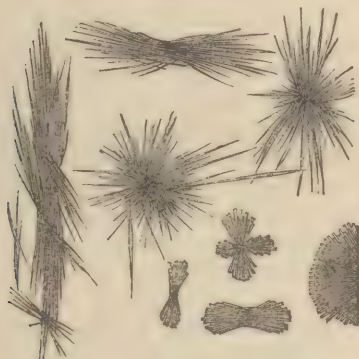
Like leucine, it is a product of the decomposition of albuminoid matters, and may be prepared from the residue of the preparation of leucine from horn. In the extraction of these compounds from the glands, the liquid is freed from albumen by ebullition, basic lead acetate is added, and the filtered solution treated precisely as indicated under leucine, except that, after all lead has been removed, the solution is evaporated and the leucine and tyrosine are separated by alcohol.

Tyrosine crystallizes in snowy-white tufts of superposed needles (Fig. 19). It is tasteless and odorless; not very soluble in cold water, but easily soluble in hot water, from which it separates almost entirely on cooling. It is insoluble in alcohol and ether, but quite solu-

ble in ammonia, in the mineral acids, and in solutions of the alkaline hydrates and carbonates.

When a boiling aqueous solution of tyrosine is treated with a solution of mercuric nitrate, as neutral as possible, a voluminous light-yellow precipitate is formed. If now a few drops of dilute fuming nitric acid be added, and

Fig. 19.

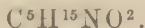


Tyrosine.

the mixture again boiled, the precipitate becomes dark red. If only a small quantity of tyrosine be present, a faint trouble will be at first perceptible, and, after boiling with fuming nitric acid, the mixture appears rose-colored and deposits dark-red flakes.

§ 63. Tyrosine may be distinguished from leucine by its crystalline form and the contraction which the crystals undergo in drying; by its difficult solubility in cold water and its insolubility in alcohol; by its decomposing when heated, while leucine may be sublimed; and by its reaction with mercuric nitrate.

Neurine or Choline.



§ 64. Neurine has been found in the bile of the pig and the ox, in the brain, and in the yolk of egg. In the latter two cases it does not exist in a free state, but in a

complex body known as lecithine, and it has thus far been found in the human body only in that form.

It may be extracted from yolk of egg or from the bile, but in this case it may result from the decomposition of lecithine (see § 72). The following process is recommended by Diakonow: Yolk of egg is exhausted successively with ether and boiling alcohol, and the residue left after the distillation of the united extracts is boiled for an hour with baryta-water. The excess of baryta is precipitated by carbon dioxide, the liquid filtered, and evaporated to dryness. The residue is extracted with absolute alcohol, the solution filtered, and precipitated by platinic chloride. A yellow double chloride which is insoluble in alcohol is precipitated; it is collected, washed with alcohol, dissolved in water, and freed from platinum by hydrogen sulphide. The filtered liquid, containing neurine hydrochloride, is evaporated to crystallization in a vacuum, and the neurine is set free by digestion with silver oxide.

The same process may be applied to the extraction of neurine from bile, which must first be freed from mucus and albuminoid matters.

Neurine is a syrupy liquid having a strong alkaline reaction and marked basic properties. Its aqueous solution is decomposed by boiling into glycol, trimethylamine, and ethylene oxide. Solutions of neurine hydrochloride are precipitated pure yellow by auric chloride, the double salt formed being difficultly soluble in cold water, and crystallizing from boiling water in small yellow needles or rhombic laminae. With platinic chloride, neurine forms a double salt which is insoluble in alcohol, but crystallizes from water, by spontaneous evaporation, in large, orange-red, oblique-rhombic prisms.

Neurine is trimethyl-hydroxethylene-ammonium hydrate.

Taurine.



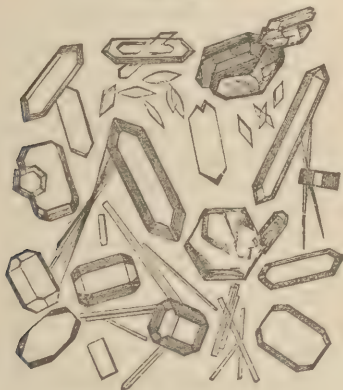
§ 65. In the human economy, taurine is found in the bile and in the intestinal canal, where it exists as a decomposition product of taurocholic acid. It is produced

by the decomposition of the latter acid by acids, alkalies, or by putrefaction. Its presence has also been demonstrated in the muscular juices and in the liquid of the lung tissues.

Taurine is most easily extracted from ox-gall. This is boiled for several hours with dilute hydrochloric acid, filtered, and the filtrate evaporated to dryness. The residue is treated with absolute alcohol to remove glycocol hydrochloride, and then dissolved in water and allowed to crystallize. The product may be purified by solution in very dilute alcohol, and precipitation by neutral lead acetate; the filtered liquid is freed from lead by hydrogen sulphide and another filtration; it may then be evaporated to dryness, washed with absolute alcohol, and recrystallized in water.

Taurine forms large, brilliant, oblique-rhombic prisms (Fig. 20), soluble in 15 or 16 parts of cold and in much

Fig. 20.



Taurine.

less boiling water, insoluble in absolute alcohol and in ether. The crystals melt when heated, and begin to decompose at 240° .

When heated on platinum foil they increase in volume and disengage sulphurous oxide, leaving a residue of difficultly combustible charcoal. If taurine be calcined

with sodium carbonate, sodium sulphide is formed, and the mass disengages hydrogen sulphide when moistened with acids.

Taurine is amid-isethionic acid.

Glycocholic Acid.



§ 66. As a sodium salt, glycocholic acid is a constant and important constituent of the bile of herbivorous animals: it exists abundantly in ox-gall, but in much smaller proportion in human bile. Traces of it have been found in icteric urine. It may be prepared by decolorizing ox-gall by animal charcoal, evaporating to dryness, and dissolving the dry residue in absolute alcohol. Ether is then carefully poured on the surface of the solution, so that the two liquids may not mix. As diffusion slowly takes place between the ether and alcohol, sodium glycocholate is deposited in crystals. These are dissolved in water, and on the addition of dilute sulphuric acid the glycocholic acid separates in fine needles, which are only slightly soluble in cold water, more soluble in boiling water and in alcohol, insoluble in ether. It does not crystallize from its alcoholic solution, but on evaporation is left as a resinous mass.

Its solutions are dextrogyrate.

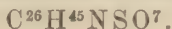
In examinations for glycocholic acid, the pure acid must be obtained. It is distinguished from cholic acid by its crystalline form, and by the fact that the latter acid contains no nitrogen. Glycocholic acid always crystallizes in fine needles.

§ 67. If an aqueous solution of glycocholic acid be mixed with two or three drops of solution of sugar, and concentrated sulphuric acid be gradually added, taking care that the mixture does not become heated, the liquid assumes first a cherry-red, then a purple-violet color. The sulphuric acid should be absolutely pure. This reaction is not peculiar to glycocholic acid, but is common to all the biliary acids (Pettenkofer).

When glycocholic acid is long boiled with dilute hydrochloric acid, or with baryta water, it is decomposed by

hydration into glyocol ($C^2H^5NO^2$), and cholic acid ($C^{24}H^{40}O^5$).

Taurocholic Acid.



§ 68. This acid exists in ox's bile, and is abundant in human bile; it is found alone in that of the dog, and has been detected in the bile of many animals, fishes, and serpents. It always occurs as an alkaline salt, and its sodium compound may be extracted from the ethereal solution from which sodium glycocholate has deposited.

The acid has not yet been obtained pure. It forms fine, silky needles, which rapidly deliquesce in the air. It is soluble in water and alcohol, insoluble in ether. Its solutions are dextrogyrate. When its aqueous solution is boiled with dilute acids, alkalies, or even alone, it breaks up into cholic acid and taurine.

Taurocholic acid is best extracted from dog's bile by the process adopted for the preparation of glycocholic acid. The crystals deposited by the ether are dissolved in a little water, and treated with basic lead acetate and ammonia. The precipitate is washed, suspended in alcohol, decomposed by hydrogen sulphide, and the filtered liquid is concentrated to a small bulk, and anhydrous ether added. The precipitate which is formed, gradually becomes transformed into silky needles, which are deliquescent in the air.

§ 69. Taurocholic acid is separated from glycocholic and cholic acids by the addition of neutral lead acetate to the *neutral* mixture. Lead cholate and glycocholate are insoluble, while the taurocholate remains in solution. The filtered liquid is mixed with basic lead acetate and ammonia; the precipitate formed is suspended in alcohol, and may be decomposed by hydrogen sulphide, or it may be mixed with sodium carbonate, the mixture evaporated to dryness, and extracted with alcohol, which dissolves only sodium taurocholate.

Taurocholic acid gives the reaction indicated in § 67, and if it be heated with sodium carbonate and nitrate, the residue will be found to contain sulphur.

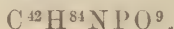
Cholic Acid.

§ 70. Traces of cholic, or as it is sometimes called, cholalic acid, are found in the contents of the small intestine and in icteric urine; it exists in larger quantity in the large intestine and in the excrements.

It is a product of the decomposition of glycocholic and taurocholic acids, and may be easily prepared by boiling for twenty-four hours in saturated baryta water the precipitate produced by ether in an alcoholic solution of bile. The barium cholate formed is then washed, decomposed by hydrochloric acid, and the cholic acid set free is purified by several crystallizations in alcohol.

It crystallizes in transparent and colorless tetrahedra, which soon become opaque in the air. It is almost insoluble in water, readily soluble in alcohol, less so in ether. It separates from boiling alcohol in quadratic crystals, containing 5 molecules of water of crystallization; from ether in four-sided prisms, containing 2 molecules of water.

§ 71. If a small quantity of cane-sugar and a few drops of concentrated sulphuric acid be added to an aqueous solution of cholic acid, taking care to add the acid drop by drop so that the liquid does not become heated, a precipitate is first formed, but is redissolved by an excess of acid. On heating the mixture to about 70° , it assumes a cherry-red color, which changes to purple, and gradually becomes darker, until in a week's time, it is violet or blue (Pettenkofer). This reaction is common to the other biliary acids, from which, however, cholic acid is distinguished by its crystalline form, and by its solubility in ether.

Lecithine.

§ 72. Lecithine has been found in most of the cellular liquids, both animal and vegetable, and notably in the blood, exudations, and bile. It exists in considerable quantity in the brain, nerves, and in the yolk of egg.

The latter substance serves well for its preparation. Yolk of egg is exhausted with a mixture of alcohol and ether, and an alcoholic solution of cadmium chloride is added to the filtered liquid. A white, flocculent precipitate, a compound of cadmium chloride and lecithine hydrochloride, is thrown down; this is washed with alcohol and ether, then suspended in water and decomposed by hydrogen sulphide. The filtered solution is evaporated, and deposits lecithine hydrochloride as a wax-like mass. When the alcoholic solution of this compound is decomposed by silver oxide, lecithine is set free, and after filtration and evaporation remains as a translucent homogeneous mass.

Lecithine and all of its compounds are very alterable. It decomposes slowly in the cold, rapidly at a moderate temperature, and even when its alcoholic solution is boiled for some time.

When an alcoholic solution of lecithine hydrochloride is boiled with baryta-water, barium oleate and palmitate are precipitated, and barium phosphoglycerate and neurine remain in solution (see § 64).

§ 73. PHOSPHOGLYCERIC ACID, $C^3H^9PO^6$.—This has been said to exist in the brain, nerves, and bile, but it is probably formed by the decomposition of lecithine, during the chemical operations employed for its preparation.

Cystine.



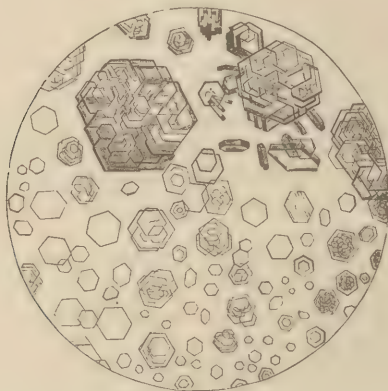
§ 74. Cystine is found in rare renal and vesical calculi, and as a sediment in pathological urine.

It may be prepared by dissolving cystic calculi or cystic urinary sediment in ammonia, and allowing the solution to evaporate. Cystine is then deposited in colorless hexagonal tables (Fig. 21) or rhombohedra; these crystals sometimes assume the forms of uric acid (*b, b*, Fig. 22), from which, however, they may be readily distinguished by their solubility in ammonia.

Cystine is insoluble in alcohol, water, and ether, but dissolves readily in alkaline solutions and in mineral

acids ; it is precipitated from the former by acetic acid, and from the latter by ammonium carbonate.

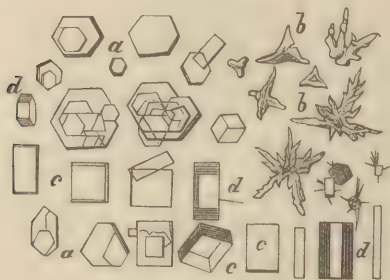
Fig. 21.



Cystine.

If cystine, or a portion of a cystic calculus, be dissolved in a small quantity of an alkaline solution, and the liquid be diluted with water and potassium nitroferrocyanide added, a rich violet color is produced (J. Muller).

Fig. 22.



Cystine.

When cystine is boiled with potassium hydrate, ammonia is disengaged, and an alkaline sulphide is formed ;

if, then, a substance containing cystine be boiled with a solution of lead oxide in potassium hydrate, a black precipitate of lead sulphide is thrown down.

Cystine is also readily recognized by its crystalline form as seen under the microscope. The suspected calculus or deposit is dissolved in ammonia; a few drops of the solution are placed on a microscope slide and covered with a thin glass. As the ammonia volatilizes, characteristic hexagonal tables are formed.

Albuminoid Bodies.

§ 75. With the exception of the bile, tears, and urine, the greater part of the liquids and tissues of the body are composed of substances having nearly the same chemical composition, and presenting such similar properties that it is sometimes difficult to distinguish between certain of them.

These substances, so important to the constitution of organized matter, receive the general name *albuminoid bodies*. They possess properties analogous to those of white of egg. They are solid, amorphous—excepting hemoglobin, which is crystallized—odorless, and almost tasteless. They are insoluble in alcohol, ether, and all neutral organic liquids. They are sometimes soluble in water, sometimes insoluble; their solubility may generally be traced to the presence of alkalies, acids, or mineral salts. Their solutions rotate the plane of polarized light to the left.

All of these bodies contain carbon, hydrogen, oxygen, nitrogen, and sulphur, and, disregarding traces of mineral salts from which it is difficult to completely separate them, they present nearly the same centesimal composition, which is as follows:—

Carbon	54.3
Hydrogen	7.1
Oxygen	21.0
Nitrogen	15.8
Sulphur	1.8

In contact with water they gradually decompose, and are converted into other substances; their tendency to

putrefy distinguishes them from other organic bodies, and, when decomposing, they evolve hydrogen sulphide.

When heated they swell up and decompose, disengaging ammonia, carbon dioxide, and pyrogenous products, and leaving a residue of nitrogenized carbon. If this be incinerated, a small residue of chlorides, phosphates, and sulphates of the alkaline or earthy metals is obtained.

§ 76. They dissolve in solutions of the alkaline hydrates, and are generally again precipitated from such solutions by the addition of acetic acid. The precipitate thus obtained seems to be identical, whatever may have been the albuminoid body employed. If lead acetate be added to the alkaline solution of an albuminoid body, a black precipitate is formed, indicating that the alkali has removed a portion of the sulphur from the albuminoid substance, forming a sulphide.

a) When boiled with concentrated alkaline solutions, these compounds are decomposed, yielding principally formic acid, carbonic acid, glycocol, leucine, tyrosine, and an alkaline sulphide.

b) Cold concentrated nitric acid communicates a yellow color to albuminoid substances, and the color is rendered more intense by warming; it is darkened to orange by the addition of ammonia.

c) Concentrated hydrochloric acid dissolves them, especially by the aid of heat, and the liquid assumes a blue or violet color if the reaction take place in contact with the air.

d) Iodine colors them intensely yellow, and serves for their recognition under the microscope.

e) They assume a red color when heated with Millon's reagent (acid nitrate of mercury);* and this test is so delicate that it will indicate the presence of $\frac{1}{100,000}$ of albumen in aqueous solution.

§ 77. *a)* Albuminous solutions are generally coagulated by boiling unless the liquid be alkaline, and, in

* Millon's reagent is prepared by dissolving, by the aid of heat, 1 part of mercury in 2 parts of nitric acid of specific gravity 1.42 and boiling at 120°. When the mercury is entirely dissolved, 2 volumes of water are added to 1 volume of the solution, and the mixture is allowed to stand twenty-four hours. The clear liquid may then be decanted from the deposit which has formed.

this case, the coagulation may be brought about by the addition of a few drops of nitric acid.

b) Albuminoid bodies are also precipitated from their aqueous solutions by mineral acids, acetic acid, and concentrated solutions of alkaline salts, alcohol, solutions of tannin or of phenol, and by the salts of the heavy metals, such as lead basic acetate, mercuric chloride, cupric sulphate.

§ 78. For the detection of an albuminoid substance of undetermined nature in a liquid, one or two general reactions are employed, and any positive results obtained may afterwards be confirmed if necessary. A portion of the liquid is boiled, and rendered acid by the addition of a little nitric acid; if a precipitate be formed by boiling and be not dissolved by the nitric acid, or if the nitric acid occasion the formation of a precipitate, it may be assumed that an albuminoid substance is present. Neither the formation of a precipitate by boiling alone, nor after the addition of nitric acid unaided by heat, can be regarded as conclusive evidence of the presence of albuminoid matter. Thus, in urine, a precipitate formed by boiling, and redissolved by a few drops of nitric acid, is probably due to the presence of earthy phosphates, while a large proportion of uric acid may give rise to the formation of a precipitate on the addition of nitric acid unaided by heat; in the latter case, the precipitate is redissolved by boiling. In any case, a large excess of nitric acid is to be avoided, since the albumen might be redissolved by that reagent.

§ 79. An albuminoid substance may also be detected by acidifying the liquid with acetic acid, adding concentrated sodium sulphate solution in volume equal to that of the suspected liquid, and heating to boiling; or the liquid, previously acidified with acetic acid, may be saturated with crystals of sodium sulphate and then boiled. The formation of a precipitate is indicative of the presence of albumen. This method gives satisfactory results.

§ 80. Albuminoid substances are generally entirely separated from their solutions by the addition of strong alcohol in sufficient quantity. If the solution be alkaline, it must first be acidified with acetic acid, and is

then allowed to stand a few hours after the addition of the alcohol.

By the action of oxidizing agents, such as potassium permanganate or chromic acid, albuminoid substances yield acids of the fatty series, acetic, propionic, butyric, valeric, and caproic, the corresponding aldehydes and nitriles, benzoic aldehyde, benzoic acid, etc.

Gastric juice, natural or artificial, dissolves them, converting them into peptones, and it seems that in this reaction a peculiar modification of albumen, syntonin, is first formed.

§ 81. Schützenberger classifies the albuminoid bodies as follows, and this classification is well adapted for the study of their more marked differences:—

Soluble in water.	Coagulated by heat.	Alone.	<i>Albumen of white of egg and of serum.</i> —Precipitated neither by acetic acid nor by orthophosphoric acid.
			<i>Vitellin.</i> —The albumen of yolk of egg. Apparently a mixture of albumen and casein.
	In the presence of acetic acid.		<i>Globulin.</i> —Found in the red corpuscles. Combined with hematin forms hemoglobin.
<i>Hemoglobin or hematocrystallin.</i> Crystallizable.			
Not coagulated by heat.			<i>Hydropisin.*</i> —Found in serous effusions (pleurisy, ascites). Differs from albumen only by being insoluble in a saturated solution of magnesium sulphate. Colored red by chlorine water.
			<i>Pancreatin.*</i> —Exists in the pancreatic juice. Insoluble in a saturated solution of magnesium sulphate; colored red by chlorine water. Possesses the property of rendering fats, starch, and albuminoid matters fit for assimilation (Generally classed as a ferment.)
			<i>Paralbumen.*</i> —A very viscous liquid found in ovarian cysts. Coagulates in flakes when its aqueous solution is boiled with acetic acid and potassium ferrocyanide.
			<i>Metalbumen.*</i> —Found in ascites. Is not precipitated by potassium ferrocyanide, and its solution is only slightly troubled by boiling with acetic acid.
			<i>Casein of milk and legumin.</i> —Alkaline albuminates; coagulated by rennet; precipitated by both acetic and orthophosphoric acids.
			<i>Soluble ferments.</i>
			<i>Peptones or albuminose.</i> —Formed by the action of gastric juice on albuminoid matters. Not precipitated by acids. Precipitated by mercuric chloride.

* Distinctive characters not well marked.

Insoluble in water.	{ Insoluble in solution of potassium nitrate or in water containing $\frac{1}{1000}$ of hydrochloric acid.	}	Coagulated albumen. Cooked fibrin.
	{ Soluble in solution of potassium nitrate.	}	Fibrin of blood.
	{ Soluble in water containing $\frac{1}{1000}$ hydrochloric acid.	}	Myosin. Gluten and Syntonin.
	{ Soluble in alcohol	}	Glutine.
	{ Characters not well-marked; distinguished from other albuminoid bodies by not assuming a blue or violet color by the action of hydrochloric acid and air.	}	Ichtidin. Ichtulín. Emyidin.

Albumen.

§ 82. The albumen of serum of blood, sometimes called *serin*, is one of the most important constituents of the animal body; it is an essential and constant element of all the nutritive fluids. Thus it exists in the blood, chyle, lymph, in all of the serous fluids, in the muscles, and cellular tissue. Pathologically it is found in various effusions, in pus, urine, etc.

In the liquids and organs of the economy, albumen always occurs in the liquid state, being probably held in solution in water by the presence of a small proportion of alkali. In this condition it very much resembles the albumen which constitutes the white of egg. Aqueous solutions of both substances deviate the plane of polarized light towards the left, but the rotatory power of egg albumen is inferior to that of serin.

a) When a solution of albumen is heated, it becomes slightly turbid at 70° , and when the temperature reaches 73° , the albumen coagulates either in a solid mass or in flakes, according to the concentration of the solution. Should the solution be alkaline, a part or the whole of the albumen may remain dissolved; but if the alkali be previously neutralized by the requisite quantity of acetic acid, the whole of the albumen will separate in large flakes on boiling. Care must be taken not to add too much acid, otherwise the coagulation may be entirely prevented.

Albumen thus coagulated is insoluble in water, alcohol, ether, and in cold dilute acids. By long boiling it is dissolved by acetic acid, and readily by hydrochloric

acid, the solution in the latter case assuming a dark, purple color.

Nitric acid always produces a white precipitate in solutions of albumen, but this precipitate is soluble in a large quantity of nitric acid, and in an excess of water.

b) An alcoholic solution of phenol precipitates albumen, even when only minute traces are present. A solution of phenol which gives good results is composed as follows :—

Crystallized phenol . . .	1 part by weight.
Crystallizable acetic acid . .	1 “ “ “
Alcohol (90 per cent.) . .	3 “ “ “

10 cubic centimetres of this solution dissolve without producing a cloud in 100 c. c. of water or other liquid containing no albumen. (Méhu.)

c) Metaphosphoric acid completely precipitates albumen from its solutions, and is one of the most delicate tests that can be employed. Ordinary phosphoric acid is without effect.

EGG-ALBUMEN.

§ 83. The albumen of white of egg closely resembles that from the serum of blood, but is not precipitated from its solutions by agitation with ether, as is the case with serum-albumen. As has been seen, serum-albumen is soluble in concentrated hydrochloric acid; when water is added to this solution a precipitate is at first formed but is easily dissolved by an excess of water. Egg-albumen is more difficultly soluble in hydrochloric acid; the solution is precipitated by the addition of water, but the precipitate is not readily redissolved by an excess of that liquid.

Egg-albumen injected into the veins or under the skin of animals, passes unchanged into the urine: this is not the case with serin.

ESTIMATION OF ALBUMEN.

§ 84. The proportion of albumen present in any liquid can only be estimated by coagulation, drying, and weigh-

ing. The coagulation may be effected by boiling, but may often be more conveniently brought about by the alcoholic solution of phenol before mentioned. Should only a small quantity of albumen be present, 100 cubic centimetres of the liquid to be examined, are filtered, acidified with 1 or 2 c. c. of nitric acid, and introduced into a stoppered bottle together with 10 c. c. of the phenol solution. The bottle is then closed, and well shaken in order to break up the coagulated albumen so that it may be readily washed. The liquid is then filtered through a tared filter, the precipitate thoroughly washed with boiling water charged with phenol, dried at 100° and weighed. Should the liquid contain a large proportion of albumen, a smaller quantity (10–50 c. c.) is taken and diluted to 100 c. c. with distilled water, the treatment then being the same as before. (Méhu.)

§ 85. There are many bodies closely allied to albumen.

VITELLIN is extracted from yolk of egg, by washing the latter with ether; it appears to be only a mixture of albumen and casein.

GLOBULIN is the coagulable principle of the red blood-corpuscles; it much resembles albumen, but is distinguished from it by being precipitated by carbon dioxide, while albumen is not. It is believed to be a product of the decomposition of hemoglobin. It, or an analogous body exists in the crystalline lens. Schmidt and Hoppe-Seyler consider it identical with the fibrino-plastic substance (see farther on).

HYDROPSIN has been found in the effusion of ascites; it differs from albumen only by its insolubility in solution of magnesium sulphate.

PANCREATIN exists in the pancreatic juice; it is colored red by chlorine, and has the property of converting starch, fats, and albuminoid bodies into digestible substances.

PARALBUMEN has been found in ovarian cysts: it is precipitated when heated with acetic acid and solution of potassium ferrocyanide.

METALBUMEN, which has also been encountered in dropsical effusions, is not precipitated when heated with potassium ferrocyanide, and only produces a faint cloud with acetic acid.

Hemoglobin.

§ 86. Hemoglobin, which has also been called hemato-crystalline, is the coloring matter of the blood, and seems to be a combination of hematin and globulin.

It may be prepared by triturating clotted blood with its own volume of water, and filtering through a cloth. The liquid is then frozen, or agitated with ether until the corpuscles are entirely dissolved. A coagulum is so formed, which removes all of the unbroken corpuscles from the liquid: the latter is then filtered, slightly acidulated with acetic acid, and alcohol is added as long as the precipitate formed continues to redissolve. The red liquid is cooled to 0° , and after a time deposits crystals, which are collected on a filter, pressed, and washed with dilute alcohol and water, both at 0° . They may be purified by recrystallization by dissolving them in water, and evaporating the solution in a vacuum, or by adding alcohol to it and cooling the mixture to 0° .

These crystals differ in form and in solubility, according to the animal from whose blood they are obtained. Human blood, and that of the horse, fish, hedge-hog and dog, yield prismatic crystals, while those obtained from the guinea-pig and mouse are tetrahedra, and are more difficultly soluble.

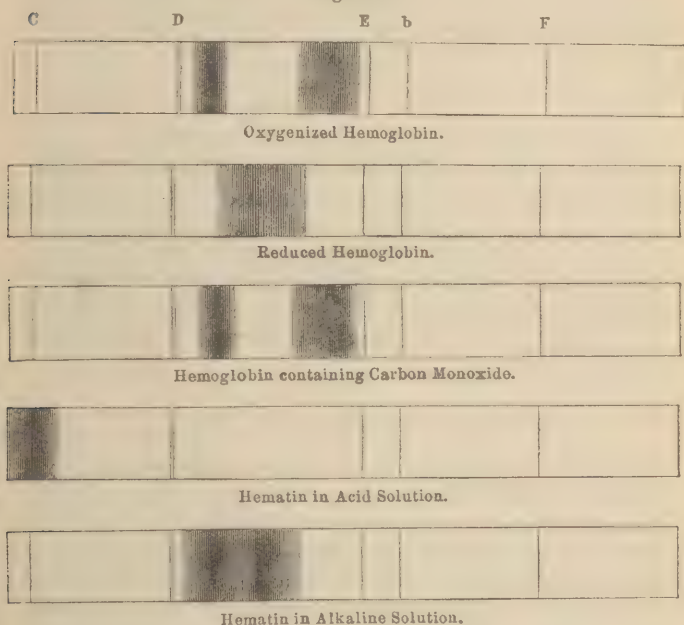
Aqueous solutions of hemoglobin coagulate at 64° , and the crystals alter rapidly on contact with air. Alkalies and acids decompose them into hematin and globulin.

Crystals of hemoglobin which have been dried in a vacuum at a low temperature, rapidly absorb oxygen and are converted into *oxyhemoglobin*. This compound exists in the red corpuscles of arterial blood; it partially loses its oxygen in a vacuum, and entirely when submitted to the action of reducing agents, such as ammonium sulphide. Venous blood contains both oxidized hemoglobin and

reduced hemoglobin. When the latter is agitated with air, it again absorbs oxygen.

§ 87. When a solution of oxyhemoglobin is placed between the source of light and the prism of a spectroscope, the spectrum is found on examination to contain two dark bands between Fraunhofer's lines D and E. The darkest and most distinct is nearer D, the other is wider and less clearly marked (see Fig. 23). These bands are characteristic of oxyhemoglobin, and it is not necessary that the latter shall be isolated, natural blood answering perfectly for the study of the bands.

Fig. 23.



If a few drops of ammonium sulphide be added to the blood, and the mixture be heated to 30° or 40° , and its absorption spectrum again examined, the dark bands will be found to have disappeared, and in their place is seen a single band wider than either of the first, and in a

position which would have been between them. This absorption spectrum is characteristic of reduced hemoglobin. These properties permit the detection of very small quantities of blood, and are of great service in legal chemistry.

Hemoglobin also combines with hydrocyanic acid and with carbon monoxide, the absorption spectrum being in each case characteristic.

HEMATIN.

§ 88. Hematin is a product of the decomposition of hemoglobin. It may be prepared by agitating defibrinated blood with ether, adding acetic acid, again shaking for some time, and filtering the ethereal solution which separates. After standing for some time, the latter deposits hematin, which is collected and washed with alcohol and ether.

It is reddish-brown, amorphous, insoluble in water, alcohol, ether, and chloroform, but dissolves in acids and alkalies. Its solution, acidulated with acetic acid, gives a wide absorption band in the spectrum, replacing the line C. Its alkaline solutions give two absorption bands which merge together between D and E. These bands are also produced by acid or alkaline blood (see Fig. 23). After calcination, hematin leaves 12.8 per cent. of ferric oxide.

HEMATIN HYDROCHLORIDE (HEMIN).

§ 89. When a little hematin or a drop of blood is placed on a microscope slide, and treated with acetic acid and sodium chloride, small, flattened, rhomboidal crystals, having acute angles, are formed (Figs. 24 and 25). These crystals are hemin, and have been regarded as crystallized hematin, and as hematin hydrochloride. They have a reddish-brown color, and are insoluble in water, alcohol, and ether, but soluble in concentrated alkaline solutions; the solution is precipitated by acids, and by alkaline and earthy salts. This reaction is of great value in medico-legal researches for the recognition of blood-stains.

§ 90. HEMATOIDIN, which has been found in old blood effusions, is a decomposition product of hemoglobin. It

Fig. 24.



Fig. 25.



crystallizes in small, orange-red, transparent prisms. It is insoluble in water and alcohol, slightly soluble in ether, soluble in chloroform. It contains no iron (see bilirubin).

Fibrin.

§ 91. Blood which is drawn from the body coagulates spontaneously to a gelatinous mass, which gradually contracts as the serum is expressed, and becomes a network of elastic filaments. This coagulation is due to the production of fibrin; and by beating the blood with a bundle of wires or twigs, the fibrin may be removed, as it is formed, in white, elastic filaments, which adhere to the rods.

On drying, fibrin becomes hard and brittle, but again becomes elastic when immersed in water. It is insoluble in water, but dissolves in alkaline solutions, forming alkaline albuminates. Hydrochloric acid containing $\frac{1}{1000}$ acid, dissolves it, forming syntonin. It dissolves at about 40° in solutions of potassium nitrate, sodium sulphate, and in ten per cent. solutions of common salt. These solutions coagulate when heated.

§ 92. Fibrin does not exist already formed in the blood; it is produced by the combination of two substances which have been named *fibrinogen* and the *fibrinoplastic substance*; these bodies do not react upon each other in the vessels, but by causes which are not understood, combine as soon as the blood is drawn, and so form fibrin. According to Schmidt, the two substances may be isolated from each other as follows: Horse's

blood is received in a saturated solution of sodium sulphate, the globules are separated by decantation, and carbon dioxide is passed through the liquid. A flocculent precipitate is formed, and is collected on a filter. After these flakes, which constitute the fibrinoplastic substance or *paraglobulin* (globulin) are removed, blood is no longer coagulable. If the current of carbon dioxide be long continued, viscous masses separate, and adhere to the sides of the vessel: these constitute the fibrinogen.

These two substances are insoluble in boiled water, or in water charged with carbon dioxide, but they dissolve in aerated water and in solutions of the alkaline hydrates and carbonates. Their solutions are not coagulable, even by heat; but when the two solutions are mixed together, coagulation immediately takes place, and fibrin is formed.

Certain pathological fluids, such as the liquid of hydrocele, do not coagulate spontaneously, but they contain fibrinogen, for they immediately coagulate in a solid mass when fibrinoplastic substance is added. Fibrinogen may be readily obtained by passing carbon dioxide through the liquid of hydrocele.

Myosin.

§ 93. This albuminoid substance exists in the liquid naturally contained in the sheaths of the muscles. This muscular juice is a thick, milky liquid which solidifies spontaneously in gelatinous masses of myosin. It also coagulates spontaneously after death, thus producing cadaveric rigidity.

It is insoluble in water, and in a saturated solution of sodium chloride. It may be prepared from the muscles, by finely dividing them, washing the mass with water until it is decolorized, and then triturating with common salt, and enough water to make a 10 per cent. solution of the salt. Myosin is soluble in such a solution, and, after cold digestion for a few hours, the mixture is filtered, and solid rock salt introduced into the liquid. As the salt dissolves, the myosin is precipitated, not being soluble in saturated solutions of sodium chloride. Very

dilute hydrochloric acid dissolves myosin, converting it into syntonin.

Syntonin.

§ 94. Syntonin, which has also been described under the names musculin and parapeptone, may be prepared from muscular tissue. It appears to be the first product of the action of gastric juice, or of very dilute hydrochloric acid upon albuminoid bodies. It is doubtful whether it pre-exists in the muscles, for it is probably formed from myosin by the method employed for its extraction.

The muscular tissues are hashed, thoroughly washed with water, and triturated with water containing one thousandth part its weight of hydrochloric acid. The meat swells, and dissolves to a considerable extent; the mixture is pressed through a cloth, the liquid filtered, and exactly neutralized with sodium carbonate. The syntonin separates in colorless, gelatinous flakes, which dry upon the filter in elastic films. It may be prepared from fibrin, or from albumen, by the same process.

Syntonin is entirely insoluble in water, but dissolves in water containing one-tenth per cent. of hydrochloric acid, or one per cent. of sodium carbonate. These solutions are not coagulated by heat, but are precipitated by water, sodium or magnesium sulphate, sodium chloride, etc. By the action of dilute sulphuric acid, syntonin yields leucine and ammonium sulphate.

ALBUMINOSE OR PEPTONES.

§ 95. Albuminoid bodies are dissolved by gastric juice, and the solutions contain substances which have been named peptones: the latter differ, according to the albuminoid body from which they are derived, but their differences are only slightly marked. They possess the common properties of dissolving in water, of being precipitable by mercuric chloride, and of passing through membranes by osmose. Before being absorbed in the economy, albuminoid bodies are converted into peptones, passing first through the intermediate form, syntonin.

Casein.

§ 96. Casein exists in solution in milk, from which it is separated in the solid state by various reagents, particularly on the addition of whey or acids. The same coagulation is brought about by the spontaneous formation of lactic acid by the fermentation of the lactose. Acetic acid, ordinary phosphoric, and tartaric acids may be added to milk in considerable quantities without producing coagulation.

Casein appears to be an alkaline albuminate, for the precipitate formed on the addition of an acid to its solutions, is identical with albumen. If solution of potassium hydrate be added drop by drop to white of egg, beaten up with its volume of water and reduced by evaporation at 40° to its original volume, the mass sets in a jelly. If this be thoroughly washed with a large quantity of water to remove the excess of alkali, and be then dissolved in warm water, a solution is obtained which presents all the characters of solutions of casein. It is precipitated by acetic acid, and the precipitate appears to be identical with coagulated casein.

Coagulated casein is white, amorphous, very soluble in alkalis, alkaline carbonates, potassium nitrate, and sodium phosphate. It exhibits the general properties of albuminoid substances.

Animal Ferments.

§ 97. The saliva, gastric juice, and pancreatic juice, contain peculiar and active ferments, which are analogous to the albuminoid bodies, but have the property of producing chemical transformation.

(a) PTYALIN is the active principle of the saliva. Its aqueous solution rapidly converts starch into glucose; in this respect it resembles diastase, a ferment derived from the vegetable kingdom, and it is not improbable that the two substances are identical.

Ptyalin may be prepared in a sufficiently pure state, by acidulating the saliva with ortho-phosphoric acid, and then adding enough lime-water to render the liquid alka-

line. Calcium phosphate is thrown down, and carries with it the ptyalin, together with albuminous substances; the precipitate is collected on a filter and washed with a little water, which dissolves out the ptyalin. On the addition of alcohol to the filtered liquid, the ptyalin is precipitated in light white flakes, which are converted into a yellowish powder when dried in a vacuum. This may be further purified by dissolving it in water, and reprecipitating by alcohol; and if the operation be repeated several times, all albuminoid matter is removed, and the resulting ptyalin is almost white, and burns without leaving a mineral residue.

(b) PEPSIN exists in the gastric juice, and is prepared from the mucous membrane of the stomach. It is soluble in water and in dilute acids, and its solution in dilute hydrochloric acid is capable of digesting albuminoid bodies, converting them into peptones.

The preparation of pepsin is analogous to that of ptyalin, but the precipitated pepsin is not readily removed by water from the calcium phosphate. Hence the process must be somewhat modified. Brücke recommends the following method: the mucous membrane of the stomach is carefully cleaned, detached from the muscular coat, and digested at 58° in water acidulated with one-twentieth of sulphuric acid, until the greater part of the mass is dissolved. The liquid is filtered, and the filtrate neutralized with lime-water: the precipitated calcium sulphate carries with it the pepsin, and the precipitate is collected on a filter, and washed with a little water. It is then dissolved in dilute hydrochloric acid, and the solution is gradually mixed with a solution of cholesterin in four parts of alcohol and one part of ether. The liquids are agitated together, and the precipitated cholesterin carries down the pepsin. The precipitate is collected, and washed first with very dilute acetic acid, then with water until all hydrochloric acid is removed. The cholesterin is then separated by shaking up the aqueous liquid containing the precipitate with ether, and the aqueous solution of pepsin is allowed to evaporate spontaneously. So obtained, pepsin is a grayish-white, amorphous powder, soluble in water and dilute acids.

The method of Wittich is the best for the preparation of a solution of pepsin which is intended to keep for any length of time. The well-cleaned mucous membrane is macerated with alcohol, and then scraped, dried, and pulverized. The powder is macerated with glycerin, which dissolves the pepsin; and the latter may at any time be precipitated by the addition of alcohol.

(c) The pancreatic juice probably contains three ferments, one of which converts starch into glucose; another transforms albuminoid bodies into peptones; and the third decomposes fats into fatty acids and glycerin (Danilewski).

All of these bodies are colorless solids when pure; they are amorphous, and are precipitated from their solutions by alcohol and lead acetate. They are not precipitated either by tannin or by mercuric chloride, nor are they colored yellow by nitric acid, as are albuminoid bodies. Very small quantities of these ferments are sufficient to effect the peculiar fermentations for which they are adapted, of an almost indefinite quantity of the fermentable body, provided the products of the fermentation be removed from the liquid as they are formed; for the presence of these products frequently impedes the action of the ferment. The activity of these ferments varies with the temperature; that of the human body, about 35° , being most suitable for their action; too high a temperature—in every case below 100° —destroys the power of the ferment forever.

Substances resembling Albuminoid Bodies.

§ 98. Certain substances are related to the albuminoid bodies by their general chemical reactions, but differ somewhat from those bodies in their centesimal composition. They are colored violet by strong hydrochloric acid. They contain approximately—

Carbon	50.0
Hydrogen	6.6
Nitrogen	16.8
Oxygen	26.6

The bodies forming this group do not greatly differ from each other.

OSSEIN.

The bones contain a cartilaginous matter which may be extracted by dissolving out the earthy salts by hydrochloric acid diluted with about nine parts of water. When the mineral matter is entirely removed, the ossein remains as a soft, elastic substance, preserving the form of the bone. It may be obtained from all tissues which furnish gelatin when boiled with water, such as the skin, cellular tissue, serous membranes, etc. Ossein soon decomposes when left to itself, but by the action of tannin and certain metallic salts, it is rendered unchangeable.

GELATIN.

All matters which contain ossein yield by long boiling with water a solution which sets in a jelly on cooling. When dry, gelatin is a colorless or yellowish, transparent and vitreous solid; it is brittle and sonorous, unaltered by the air. In cold water it swells, and dissolves to a slight extent; boiling water dissolves it readily, and the solution gelatinizes on cooling. It is insoluble in alcohol. It is not precipitated by acids, by alum, or by the salts of lead, copper, silver, etc., but is thrown down by solutions of mercuric chloride.

Tannin precipitates gelatin from its solutions. When boiled with dilute sulphuric acid, or with alkalis, gelatin yields, among other products, leucine and glycocol.

CHONDRIN.

Chondrin is prepared by boiling the cartilages of the short ribs with water; the solution gelatinizes on cooling, but the substance differs from gelatin in that its solutions are precipitated by all the acids, and by many metallic salts. Alum forms in it an abundant precipitate.

When boiled with sulphuric acid, chondrin yields leucine, but no glycocol.

KERATIN is the name given to the basis of horny tis-

sues, such as the nails and hair, or the horns and wool of animals. It is softened somewhat by boiling with water, but does not yield gelatin. It contains sulphur.

MUCIN is the substance which gives secretions of the mucous membranes their peculiar viscous, glutinous, and stringy properties.

It may be extracted from ox-gall by agitating that liquid with three or four times its volume of alcohol, and collecting on a filter the impure mucin which is precipitated. This is then redissolved in water, the solution filtered, and the mucin precipitated by the addition of acetic acid, in which it is insoluble. It is finally washed with alcohol. Mucin precipitated by acetic acid is not soluble in pure water, but if a trace of an alkaline hydrate, or carbonate, or even of an acid carbonate, be present, it dissolves readily. When precipitated by alcohol, however, it dissolves in pure water, forming a viscous solution.

Mucin may be distinguished by its being precipitated by acetic acid, an excess of which does not redissolve the precipitate.

Animal pigments.

§ 99. Numerous coloring matters exist in the body, and, with few exceptions, they are very imperfectly known. Many of those which have been described have undoubtedly been produced by the action of the reagents employed, and cannot be supposed to pre-exist in the body. With regard to many others, it has justly been said that their names are their most characteristic features.

§ 100. The brown or black pigment to which the chorioid owes its color, and which is deposited in the Malpighian bodies in the skin of animals and man, especially in the negro, has been named *melanine*. It exists also in the hair, in feathers, and in the skin of reptiles, fish, etc. Under the microscope, it appears in small granular masses which are often angular. It has not been well studied.

The pigment of the eye, skin, etc., is rapidly destroyed by alkaline solutions, and by chlorine. It may thus be distinguished from carbon, which is sometimes mechani-

cally deposited and encysted in the lungs and air passages, and which has there been sometimes mistaken for an animal pigment.

The coloring matter of the blood, hematin, has already been described (§ 88).

The biliary pigments have been well studied, and may be characterized without difficulty.

Bilirubin.



§ 101. Bilirubin is found in biliary calculi, in the bile of man and other animals (ox-gall does not contain it), and sometimes exists pathologically in the blood and urine. It appears to be identical with the hematoidin which is found in microscopic crystals in old extravasations of blood (see § 90).

Bilirubin may be prepared from biliary calculi: they are pulverized, and exhausted with ether to remove the cholesterin; the residue is boiled with water, and then treated with dilute hydrochloric acid. The bile pigments are thus set free from their earthy combinations, and the portion which remains undissolved by the acid is thoroughly washed with water, dried, and then boiled with successive portions of chloroform as long as the latter removes coloring matter. The chloroform solution is filtered, the chloroform distilled off, and the residue treated with absolute alcohol which removes the bilifuscin, but leaves the bilirubin undissolved. The latter is washed with a mixture of ether and alcohol, redissolved in chloroform, and precipitated by the addition of dilute alcohol to its solution.

Bilirubin is thus obtained in orange-colored flakes. By the evaporation of its chloroform solution, it crystallizes in small, red, oblique-rhombic prisms. It is almost insoluble in water, alcohol, and ether, but dissolves easily in carbon disulphide, benzol, and chloroform, from which solvents it is precipitated by the addition of alcohol.

It is also quite soluble in solutions of the alkaline hydrates and carbonates, forming orange-red solutions, which become green on exposure to the air. Hydro-

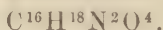
chloric acid precipitates it from its alkaline solutions. The alkaline compounds of bilirubin are insoluble in chloroform.

If concentrated sulphuric acid be added to a chloroformic solution of bilirubin, the liquid assumes a green color.

§ 102. If an alkaline, aqueous solution of bilirubin be poured on the surface of nitric acid containing a little nitrous acid, in a test-tube, or on a mixture of strong nitric and sulphuric acids, green, blue, violet, red, and yellow shades are developed at the surface of contact of the liquids. If the liquids be agitated together, the colors are produced successively in the order mentioned, but if care be taken to prevent their mixture, all of the colors may be observed at the same time (Gmelin). This reaction is characteristic and sensitive, but no alcohol should be present.

Ammoniacal solutions of bilirubin are precipitated by calcium chloride, barium chloride, lead nitrate, and silver nitrate.

Biliverdin.



§ 103. This coloring matter has not yet been certainly found in the bile or in biliary calculi, as a pre-existing compound, for it is probably formed in its extraction by the oxidation of bilirubin. It may, however, exist in the green bile of the ox, as well as in human bile which has a green color, and in icteric urine.

When a solution of bilirubin in sodium hydrate is agitated in contact with air, it assumes a green color, and hydrochloric acid precipitates biliverdin from the liquid.

It is a bright green powder, insoluble in water, ether, and chloroform, but soluble in alcohol, to which it gives a bluish-green color.

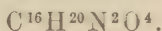
It also dissolves in glacial acetic acid, from which it separates on evaporation in green, ill-defined, rhombic tables.

With nitric acid containing a trace of nitrous acid, alka-

line solutions of biliverdin give the same series of colors as bilirubin, beginning, however, with the blue.

The green color of bile or of icteric urine cannot be considered a proof of the presence of biliverdin.

Bilifuscin.

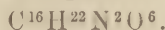


§ 104. Bilifuscin exists in very small quantity in biliary calculi.

It is prepared from the residue left after distillation of the chloroform in the preparation of bilirubin (§ 101). Alcohol extracts the bilifuscin from this residue, leaving the bilirubin undissolved. The alcoholic solution is evaporated to dryness, exhausted with ether, to remove fatty matters, and the residue washed with chloroform and dissolved in absolute alcohol. The alcoholic solution being evaporated to dryness, leaves the bilifuscin as a brilliant, almost black, porous mass, which when powdered has a dark-green color. It is only very slightly soluble in water, ether, and chloroform, but dissolves in alcohol, forming a dark-brown solution. Its very dilute alcoholic solution has the color of icteric urine.

Bilifuscin is soluble in dilute alkaline solutions, to which it gives a red-brown color, but these solutions gradually decompose on contact with the air. Hydrochloric acid precipitates them brown.

Biliprasin.



§ 105. This pigment occurs in biliary calculi, in ox's bile, and probably in icteric urine.

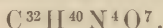
It is a brittle, brilliant black substance, which becomes dark-green when powdered. It is insoluble in water, ether, and chloroform, but dissolves in alcohol, forming a green solution which becomes brown on the addition of an alkali. It may be prepared from the residue of gallstones which have been extracted with ether, water, hydrochloric acid and chloroform.

With nitric acid containing nitrous acid, alkaline solu-

tions of biliprasin give the same reaction as those of biliverdin, but the blue zone appears only after some time.

Biliprasin is distinguished from biliverdin by the fact that its alcoholic solutions are colored brown by alkalies; from bilifuscin by the green precipitate produced by acids in alkaline solutions of biliprasin.

Hydrobilirubin or Urobilin.



§ 106. This body, which may be obtained by the action of nascent hydrogen upon an alkaline solution of bilirubin, has been detected in febrile urine. By precipitating the solution of reduced bilirubin by hydrochloric acid, hydrobilirubin is obtained in reddish-brown flakes. Hoppe-Seyler has shown that an identical compound may be prepared by the action of hydrochloric acid and tin upon the coloring matter of the blood, or upon hematin.

§ 107. The presence of hydrobilirubin in urine may be detected directly, and without preparation, by means of the spectroscope; its absorption spectrum presents an intense dark band between the green and blue, or more definitely between the Fraunhofer lines *b* and *F*; this band gradually fades towards *F*, and is nearer *b* than *F* when an alkaline solution is employed.

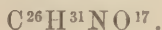
Hydrobilirubin is an amorphous, brown-red powder, very slightly soluble in water, but soluble in alcohol, ether, and chloroform. It is also soluble in alkalies, and, if zinc chloride be added to its strongly ammoniacal solution, the liquid becomes rose-colored, and acquires a green fluorescence; at the same time the absorption band becomes more intense if the liquid be spectroscopically examined.

§ 108. Hoppe-Seyler has shown that normal urine does not contain hydrobilirubin, but a compound which, when precipitated by tribasic lead acetate, and the precipitate decomposed by alcohol and sulphuric acid, gradually yields hydrobilirubin by spontaneous oxidation. This is the process described by Jaffe for the prepara-

tion of hydrobilirubin; but in highly febrile urines the hydrobilirubin may be precipitated immediately by adding zinc chloride and ammonia. The zinc compound which is deposited is collected, washed with cold and then with hot water, decomposed by sulphuric acid and alcohol, and the filtered liquid agitated with half its volume of chloroform and an excess of water. The residue of the distillation of the chloroform extract consists of hydrobilirubin.

The *urochrome* of Thudichum and the *urohematin* of Harley, seem to have been hydrobilirubin, more or less altered by the reagents used in the preparation of the pigments to which those names were applied.

Indican.



§ 109. This compound, which is identical with the *uroxanthin* of Heller, is found in small quantity in normal urine, and often abundantly in pathological urine, especially in cases suffering from cancer of the liver. Its existence in the urine was discovered by Schunk.

Indican is a substance analogous to the glucosides, and under the influence of acids is decomposed into *indigotin*, $\text{C}^8\text{H}^5\text{NO}$, and *indiglucin*, $\text{C}^6\text{H}^{10}\text{O}^6$; at the same time, *indirubin* (see further on) and other products are generally formed.

§ 110. Indican may be extracted from fresh urine as follows: albumen, if present, is removed, and the liquid is precipitated with basic lead acetate, and filtered; the filtrate is mixed with ammonia, and the precipitate which forms is collected, washed, suspended in alcohol, and decomposed by hydrogen sulphide. The lead sulphide formed is separated by filtration, and the alcoholic liquid evaporated, first on a water-bath, and finally over sulphuric acid in a vacuum.

The indican is thus obtained as a light-brown syrup, soluble in all proportions of water, alcohol, and ether.

It may be recognized by its decomposition into indigotin by boiling with hydrochloric acid.

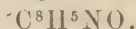
Under the influence of ferments, especially during the

putrefaction of albuminous urine, indican decomposes spontaneously, and a small quantity of *white indigo* is formed; this oxidizes into indigo-blue on exposure to the air, and the fact explains the appearance of blue pellicles having a red, metallic reflection, occasionally seen on the surface of putrid urine. Such pellicles consist of crystallized indigotin.

Urine containing large quantities of indican disclose the presence of the latter substance when boiled with hydrochloric or dilute nitric acid; a pulverulent precipitate of indigotin is immediately formed and slowly deposits. An excess of nitric acid would destroy the blue color.

Small traces of indican may be detected by mixing the urine, contained in a test-tube, with about its own volume of fuming hydrochloric acid; the presence of indican is indicated by a violet or intense blue color; the addition of two or three drops of nitric acid augments the delicacy of the test, but diminishes its permanence, the violet color changing to red and yellow (Heller).

Indigotin (indigo-blue, uroglaucin).



§ 111. Indigotin has not been found in the economy or in the excretions, but its presence is frequently observed in putrid urine, where it is formed by the decomposition of indican. It is an amorphous, dark-blue powder, or crystallized in microscopic, right-rhombic prisms, grouped in stars. When in a mass, it leaves a brilliant copper-red trace on paper. It volatilizes when heated, yielding a crystalline sublimate having a copper-red reflection.

It is insoluble in water, very slightly soluble in alcohol and ether. It dissolves in fuming sulphuric acid, forming an intense blue solution, which is immediately bleached by nitric acid.

Alcoholic solution of indigotin is also immediately decolorized by ammonium sulphydrate and other reducing agents.

The substances described as *uroglaucin*, *urine blue*,

and *urocyanin* were probably indigotin more or less pure, and of which the solubility was slightly modified by the foreign substances with which it was contaminated.

Indigo-red, indirubin.

§ 112. This substance, which was named *urrrhodine* by Heller, is amorphous, and has not been prepared in a state of purity. It may be obtained from the violet urine in which the indican has become decomposed, by filtering, adding acetic acid, and agitating the liquid with chloroform. On evaporation of the chloroform extract, the red substance remains; it is very soluble in alcohol, ether, and chloroform. It is decolorized by ammonium sulphydrate, chlorine, and alkaline hypochlorites.

PART II.

ANALYSIS OF SECRETIONS, EXCRETIONS, &c.

URINE.

§ 113. The urine is a complex liquid whose character and composition depend greatly upon the diet, manner of life, and other peculiarities of the individual. Its composition varies also with the health, and a chemical analysis of the urine is often of great service as an aid to diagnosis; but it must be remembered that the relative quantities of the normal constituents of urine, vary within certain limits even in health, so that the conclusion must not be too hastily adopted that any one is in excess or in too small a proportion.

Physical Properties.

§ 114. Normal human urine is a limpid, amber-colored liquid, having a peculiar, aromatic odor, a bitter, salty taste, and a decided acid reaction. Its specific gravity varies from 1005 to 1030, depending upon the proportions of solid and liquid food ingested, the time at which the urine is passed, and the amount of physical exercise taken by the individual. Urine which is voided soon after copious draughts of water or other liquid is generally pale in color, and has a low specific gravity; that passed after a meal has a high specific gravity, and is dark in color. The urine voided after a night's rest has a mean specific gravity, and may be regarded as representing the average composition of the total secretion for the twenty-four hours. The average specific gravity is about 1018, and the average daily amount of urine passed is about 1200 cubic centimetres, although a difference of

several hundred cubic centimetres more or less may be perfectly normal and without clinical importance.

§ 115. When allowed to stand, urine gradually deposits a light, flaky cloud of mucus, and on cooling it often deposits a sediment of which the color is variable. When allowed to stand for some time, it becomes turbid owing to the separation of crystals of uric acid and acid urates (of sodium and ammonium), and finally acquires an ammoniacal odor, and an alkaline reaction. A whitish pellicle then forms on its surface, and the bottom of the vessel containing it is covered with a white, flocculent deposit, in which crystals of ammonio-magnesium phosphate are often discernible by the naked eye. The alteration is caused by the transformation of the urea present into ammonium carbonate, and the reaction of the sodium acid phosphate, to which normal urine owes its acidity, on the urates. Acid urates and uric acid are formed, and, being but slightly soluble, are deposited, while the sodium acid phosphate is converted into neutral and basic salts.

Normal Constituents of Urine.

§ 116. The following substances are regarded as normal and constant constituents of human urine:—

Water, urea, uric and hippuric acids, creatinine, xanthine, indican, vesical mucus, sodium chloride, potassium chloride, alkaline sulphates, sodium acid phosphate, calcium phosphate, magnesium phosphate, traces of ammoniacal salts, iron, silica, and of nitrates and nitrites.

§ 117. Small quantities of calcium oxalate, succinic acid, and glucose are also found from time to time in healthy urine, but these substances cannot be considered as constantly present.

The substances formerly known as extractive matters are viscous bodies of which the chemical nature is unknown.

§ 118. Normal urine is not coagulated by boiling, nor is it precipitated by acids: if it be mixed with nitric or hydrochloric acid, its color is darkened, and in the course of twenty-four hours a precipitate of uric acid is deposited.

Abnormal Constituents of Urine.

§ 119. The abnormal constituents of urine may be classified as abnormal substances proper, such as depend upon a pathological condition of the system, and accidental constituents which are derived from certain aliments, or which pass through the system unchanged and are eliminated by the kidneys; such are the mineral poisons and many remedial agents.

§ 120. *The abnormal constituents proper*, and of which the amount present may vary greatly, are albumen, glucose, inosite, lactic acid and the lactates, fatty matters, and the volatile fatty acids, succinic and benzoic acids, salts of the biliary acids, biliary pigments, cystine, leucine, tyrosine, hemoglobin, fibrin, pus and spermatric secretion, ammonium carbonate, calcium oxalate, hydrogen sulphide.

§ 121. The urine may be turbid when voided, and may subsequently become clear by the deposition of the substances which were held in suspension; these substances then constitute the *sediment*. Sometimes the urine is perfectly limpid when passed, and deposits an abundant sediment after standing for a short time. The nature of the sediment is revealed by microscopic examination; it more frequently consists of uric acid, urates, calcium oxalate, calcium phosphate, ammonio-magnesium phosphate, mucus, epithelial cells, or sometimes of cystine, tyrosine, xanthine, pus cells, blood-globules or clots, tube casts from the kidney, spermatozooids, or infusoria.

§ 122. *The accidental constituents* of urine may be traced to the aliments or medicaments from which they are derived. The alkaline carbonates pass unchanged into the urine, and render it alkaline; the neutral alkaline salts of vegetable acids are usually eliminated as carbonates; the soluble bromides and iodides may be detected in the urine shortly after their ingestion. The vegetable alkaloids are but little changed, or not at all. The essential oils of valerian, garlic, assafoetida, etc., communicate their peculiar odors to the urine; after the ingestion of turpentine, the urine possesses an odor re-

sembling that of violets. The pigments of gamboge and rhubarb pass into the urine. Oil of bitter almonds and benzoic acid are converted into hippuric acid; asparagin and malic acid are eliminated as succinic acid.

These and analogous facts must be borne in mind in the clinical examination of urine.

Chemical Examination of Urine.

§ 123. In a chemical examination of urine for the determination of the presence or absence of any abnormal substance, or of the proportions of the normal constituents present, the urine of the whole twenty-four hours should be examined. As this is often impracticable, the chemical examination may be confined to the urine passed in the morning, before any liquid or solid food is ingested; the composition of such urine fairly represents the mean of the twenty-four hours.

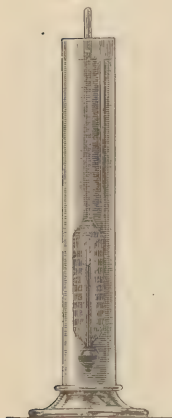
§ 124. QUANTITY.—A healthy adult excretes from 800 to 1500 cubic centimetres of urine per day, according to the age of the individual, his weight, alimentation, and the physical exercise which he undergoes.

This quantity varies greatly in disease; in certain affections the renal secretion is almost suppressed, while in the disease known as diabetes insipidus the quantity may be enormous; exceeding ten, fifteen, or twenty litres.

It has been found that there is a relation between the amount of urine excreted and the weight of the individual, so that per kilogramme of weight a healthy adult man passes about 1 c. c. of urine per hour. Hence, a man weighing 60 kilos, should eliminate about 1440 c. c. per day. This approximation is in most cases very closely in accord with the facts.

§ 125. CONSISTENCE.—Normal urine is mobile like water, and not at all viscous. When shaken in a bottle

Fig. 26.



which is half full, it froths in large bubbles which soon disappear. If the urine contain blood, pus, or biliary products, the froth is much more abundant and persistent.

§ 126. SPECIFIC GRAVITY.—The density of urine depends upon the proportion of water and solid constituents, and so in great measure upon the amount of the secretion, since the actual quantity of solid matter daily excreted by the kidneys does not greatly vary. The specific gravity is determined by means of an urinometer (Fig. 26); care must be taken that the jar in which the instrument floats be large enough that the widest part of the urinometer may not touch the sides, and the degrees of the instrument must be read from below, looking through the liquid. The urinometer should be graduated from 1000 to 1050.

The temperature of the urine should always be observed when its density is determined. A difference of 3° in temperature corresponds very nearly to one degree of the urinometer; thus a sample of urine whose density is 1024 at 15° , will have a density of 1022 at 21° .

In diabetes insipidus the density of the urine may be as low as 1001, while in saccharine diabetes it may attain 1050 or even 1070. A sample of urine having a high specific gravity should always be tested for glucose.

The density of normal urine is generally comprised between 1014 and 1030, when excessive quantities of liquid are not taken. The mean density is about 1018, this corresponding to an excretion of about 1250 c.c. per day. In warm weather, when a large proportion of water is eliminated by the skin, the density of the urine is generally increased, while it is often correspondingly lowered in winter.

§ 127. REACTION.—Normal human urine is acid; it immediately reddens blue litmus-paper. It may become alkaline if the individual be subjected to an exclusively vegetable diet, or if salts of vegetable acids, alkaline carbonates, or alkaline mineral waters, be freely administered. Urine voided immediately after a full meal is sometimes neutral or even slightly alkaline.

The acidity of the urine cannot be attributed to uric acid, or to the small quantity of hippuric acid present,

for a cold saturated solution of uric acid is almost without action on blue litmus-paper, and the boiling saturated solution produces but a wine-tint.

It is regarded as almost beyond doubt that the sodium neutral phosphate which exists in the blood reacts with the uric acid, as both are eliminated together by the kidneys, forming sodium urate and sodium acid phosphate. It is unquestionable that the latter salt is the cause of the acidity of normal urine. Free uric acid is not believed to exist in normal urine; it is almost insoluble in cold water, and would invariably be deposited as the urine cools.

§ 128. To neutralize the entire acidity of the whole amount of urine passed in twenty-four hours, from 1 to 1.5 grammes of sodium hydrate are required. It may sometimes be interesting to determine whether this normal acidity is increased or diminished; the estimation is made by neutralizing 100 c.c. of the urine with a solution of 4 grammes of sodium hydrate in 1 litre of water.

This decinormal alkaline solution is dropped from a burette into the urine until the latter becomes exactly neutral, as is indicated by its having no action on either red or blue litmus paper. Since 1 c.c. of the solution contains 4 milligrammes of sodium hydrate, the number of cubic centimetres used multiplied by 4, and by the number of 100 c.c. passed per day, expresses the daily acidity of the urine in milligrammes of sodium hydrate. The acidity may also be expressed as equivalent to a certain quantity of crystallized oxalic acid, of which 1 c.c. of the sodium hydrate solution is equivalent to 6.3 milligrammes. The acidity of the urine for twenty-four hours is equivalent to 1.6–2.4 grammes of crystallized oxalic acid.

As has been said, the daily acidity of the urine is sufficient to neutralize from 1 to 1.5 grammes of sodium hydrate; the mean daily excretion of uric acid is about 5 decigrammes; this would be sufficient to neutralize about 24 centigrammes of sodium hydrate, supposing neutral urate to be formed. The daily excretion of hippuric acid rarely exceeds 3 decigrammes, a quantity which would not quite saturate 7 centigrammes of sodium

hydrate. It is thus seen that the uric acid and hippuric acid together could in any case only account for $\frac{3.1}{18.0}$ or $\frac{3.1}{18.0}$ of the acidity of the urine.

Now the daily elimination of acid phosphates corresponds to about 2.3 grammes of phosphoric anhydride; to convert these phosphates into neutral salts of the formula R^2HPO^4 would require 1.3 grammes of sodium hydrate. The mean daily acidity of the urine does not exceed this figure. A simple calculation, based upon analyses, thus shows that the acid phosphates alone are capable of fully accounting for the acidity of the urine, the greater part of these phosphates consisting of the acid phosphate of sodium, NaH^2PO^4 .

§ 129. *Alkaline urine*.—As has already been mentioned, normal urine becomes alkaline after standing some time, the urea being converted into ammonium carbonate. This change is more rapid as the temperature is more elevated, or when blood or pus is present. Some urines which contain albumen and pus, are acid at the moment of micturition, but become alkaline in a few hours. It should not be concluded that a urine was alkaline when passed unless the test be applied immediately after micturition.

Unless the alkalinity of a urine can be traced to diet or medicament, it is an unfavorable symptom; it may be produced in the kidney when that organ is diseased, or the urine may become alkaline in the bladder. In this case it is generally turbid, thick, and even viscous at the moment of emission.

The alkalinity is due to either sodium carbonate, sodium phosphate, or ammonia; in the former case, earthy phosphates are deposited a short time after the urine is voided; when the alkalinity is produced by the spontaneous formation of ammonium carbonate, ammonio-magnesium phosphate is also precipitated.

Normal urine being acid, contains no free ammonia, and the alkalinity of an alkaline urine may readily be traced to its cause; for this purpose, a small quantity of the urine is heated in a test-tube, and a moistened red litmus-paper is suspended a short distance above its surface. Should ammonia or ammonium carbonate be pre-

sent, the paper will become blue, and a glass rod dipped in hydrochloric acid and held above the urine, will be surrounded by thick white fumes.

If these results be not obtained, the alkalinity is due to an alkaline carbonate or phosphate. In the latter case, the urine is troubled by heat, but the cloud is readily dissolved on cooling and passing a stream of carbon dioxide through the liquid.

§ 130. COLOR.—But little is known of the nature of the coloring matters of urine, and the cause of the yellow color of normal urine is not as yet understood. Healthy urine may have any of the shades of amber, and sometimes has an orange tint. When large quantities of urine are eliminated, it is usually pale, or sometimes even colorless; this is observed in disease, and after the ingestion of much beer or wine, especially champagne. In certain affections the coloring matter of the blood or bile may pass into the urine; the latter is then red, orange, greenish, brown, and sometimes nearly black. After poisoning by hydrogen arsenide, the urine is colored red by the presence of hematin.

Hydrobilirubin has been detected in febrile urine, and normal urine contains indican (see § 106 and § 109). The detection of abnormal coloring matter will be indicated farther on (§§ 136–138).

Detection of Abnormal Substances.

ALBUMEN.

§ 131. Albumen is one of the most important substances that may appear pathologically in the urine, and a determination of its absence or presence is of great service in diagnosis. Normal urine contains no albumen, but in the affection known as Bright's disease, in other renal affections, in certain fevers, and in some diseases of the spinal cord, more or less albumen is always present, and the gravity of the case may often be determined by the proportion of that substance so eliminated.

The general properties of albumen, and the means employed for its recognition, have already been de-

scribed (§§ 76-78). The nitric acid test, indicated in § 78, is conclusive, if the proper precautions be observed. The urine, contained in a test-tube, is acidified with one or two drops of acetic acid, and is then boiled for a few minutes. The formation of a cloud may be due to the separation of phosphates, or to the coagulation of a trace of albumen; the distinction is made by the addition of a few drops of nitric acid; phosphates would be redissolved, while, unless an excess of nitric acid be employed, albumen will be unaffected. If much albumen be present, the entire mass of liquid may coagulate.

By testing some of the precipitate with hydrochloric acid, as indicated in § 76, *c*, all doubt of its nature may be removed.

The separation of albumen from its solutions by means of sodium sulphate has already been described (§ 79), as has also its detection by the use of an alcoholic solution of phenol (§ 82, *b*).

In the application of any of these tests, the urine must be perfectly clear, and if it possess any turbidity, due to pus or the separation of sediment, it must be previously clarified by filtration.

The application of the nitric acid test by pouring the urine upon the surface of nitric acid in a test-glass, and examination for turbidity at the line of contact of the two liquids, is untrustworthy and cannot be recommended.

GLUCOSE.

§ 132. It cannot be admitted that normal urine contains glucose, although but few specimens of urine can be found which do not reduce Fehling's solution to a very slight extent. However, glucose is always present in the disease known as diabetes mellitus, and its quantity may then exceed even 100 grammes per litre. Diabetic urine is generally pale in color, and has a high specific gravity (1030-1050); the daily excretion is usually increased to at least double the normal average, and in some cases may reach twenty litres.

Urine containing albumen must always be freed from that substance before being tested for glucose; this is

accomplished by boiling the urine with a few drops of acetic acid, and filtration from the albumen precipitated; or the urine may be saturated with sodium sulphate, filtered, boiled, and again filtered to separate the coagulum; by this method, albumen and glucose may both be estimated quantitatively in the same portion of urine. Besides this, the urine employed should always be fresh.

Glucose is then sought by one of the tests given in sections 21-24. Of all these tests, preference must be given to that by means of Fehling's solution. The reduction of the blue liquid takes place in the cold, as well as by the aid of heat, but is then much slower, several hours or even an entire day being required.

If the urine contain only a small proportion of glucose (two or three grammes per litre), at least 1 c.c. must be added for every 5 c.c. of Fehling's solution, and the mixture must be boiled during several minutes. The reduction may be rendered more rapid by previously boiling the urine with a solution of sodium hydrate; much of the urica is thus decomposed and eliminated, and the liquid will be more prompt in its action on Fehling's solution.

In the application of the copper reduction test by Trommer's method, Fehling's solution, or in any other manner, the complete disappearance of the blue color, and its replacement by red or yellow, is indicative of the presence of glucose; the dark-red precipitate of cuprous oxide is not always formed, unless great care be taken, even though the reduction be perfect.

When a non-albuminous urine is without action on Fehling's solution, it may be safely concluded that no glucose is present.

INOSITE.

§ 133. Inosite has been found in the urine in a few cases, being sometimes associated with glucose, sometimes with albumen. It can only be detected by separating it in a pure state, and applying the characteristic tests, as indicated in sections 26-28.

LACTIC ACID (PARALACTIC).

§ 134. Paralactic acid is said to be present in diabetic urine, and in urine containing an excessive proportion of either uric acid or calcium oxalate. However, it is rarely found in fresh pathological urine. Schultzen has noticed that it appears in the urine after poisoning by phosphorus.

To detect its presence, the urine is concentrated on a water-bath, the still warm liquid is mixed with 95 per cent. alcohol, and the alcoholic extract treated as directed in § 14. The paralactic acid is recognized by the characters of its zinc salt.

BILIARY ACIDS AND PIGMENTS.

§ 135. Small quantities of the biliary acids sometimes pass into the urine; for their detection, as large a quantity as possible (about 500 c.c.) of the urine is evaporated to dryness, the residue extracted with 85 per cent. alcohol, and the extract filtered. The alcohol is distilled off, and the new residue is exhausted with absolute alcohol, the solution filtered, and again evaporated to dryness. The last residue, which is free from mineral salts, is dissolved in a little distilled water, and treated with ammonia and basic lead acetate, until the latter produces no further precipitate. In about twelve hours, the precipitate is collected, washed with distilled water, pressed between folds of filter-paper, and boiled with alcohol. The liquid is filtered boiling, and the filtrate treated with sodium carbonate, which reacts with the lead salts of the biliary acids. The mixture is then evaporated to dryness and the new residue boiled with alcohol, which dissolves the sodium compounds of the biliary acids. The alcoholic liquid is filtered, evaporated to dryness, and the residue extracted with water. The solution thus obtained may be directly submitted to Pettenkofer's test (§ 67).

It is introduced into a small test-tube and mixed with two or three drops of a solution of one part of cane-sugar in four parts of water. Pure concentrated sulphuric acid, containing no sulphurous acid, is then added drop by drop

until the liquid becomes somewhat heated, but the temperature should not be allowed to rise too high. The presence of biliary acids is indicated by a turbidity which soon disappears, the liquid then becoming yellow, cherry-red, deep red, and finally violet or purple. The reaction may take place immediately, or, if too many precautions have been taken, may not become apparent until after the lapse of some time.

§ 136. Normal urine contains no trace of biliary pigments, but in certain diseases these coloring matters pass into the blood and thence into the urine, which becomes dark yellow, brown, or green. Such urine generally yields an abundant, persistent froth when agitated, and produces dark stains on white filter-paper.

The biliary pigments are detected by Gmelin's test. The urine is introduced into a conical test-glass, and nitric acid containing nitrous acid in solution is allowed to flow slowly down the side of the glass, so that the liquids may not mix at once. Nitric acid which has become yellow by exposure to sunlight answers best for the purpose. If sufficient biliary pigment be present, colored zones will be formed at the bottom of the glass where the liquids have mixed, their colors being green, blue, violet, red, and yellow, in this order from above downwards. If bilirubin be present, the green appears first, and is then characteristic.

Gmelin's test is very delicate, and may be rendered more so by operating as follows: 2 or 3 cubic centimetres of pure nitric acid and one drop of nitric acid charged with nitrous acid, are introduced into a test-tube, and the urine is allowed to flow slowly upon the surface of the acid by the aid of a pipette. The colored zones may then appear simultaneously, or successively in the order given above. The urine tested should contain no alcohol, but the presence of albumen does not affect the sensitiveness of the test.

When mixed with hydrochloric and acetic acids, urine containing biliprasin produces a green color which changes to brown when the liquid is neutralized by ammonia. Most icteric urines produce this reaction.

§ 137. *Red hepatic urine*.—This name is applied by

Méhu to urine which assumes a violet-red color when mixed with two or three times its volume of hydrochloric acid; such urine behaves in the same manner with sulphuric acid, but nitric acid produces a mahogany or hyacinth-red. Urine possessing these properties is not unfrequently encountered. According to Méhu, its coloring matter is derived from the liver, and it seems probable that it may be hydrobilirubin in a more or less altered condition. He separates the pigment, as well as other biliary pigments which may be present, by acidifying the urine with about one gramme of sulphuric acid per litre, and then saturating the acid liquid with ammonium sulphate. When no more ammonium sulphate dissolves, the liquid is filtered, and the filtrate will be nearly colorless, while the pigment will remain upon the filter, having a brownish-yellow color. As it is soluble in water, it is dried without washing, and may be extracted by absolute alcohol.

If the mixture of urine and ammonium sulphate be allowed to stand, flakes of pigment separate and either remain floating near the surface of the liquid or are in great part deposited.

It may be remarked, with regard to this method of Méhu, that comparatively few samples of urine can be found in which more or less of a yellowish coloring matter is not precipitated by saturating them with ammonium sulphate, while at the same time the color of the liquid is much lightened. It is therefore of importance that the precipitate which may be obtained from normal urine should be studied before applying the test to abnormal urine.

Harley recommends, for the detection of abnormal coloring matter *derived directly from the blood*, that the urine be boiled, acidulated with nitric acid, and when cold agitated with ether; the ether removes the coloring matter, and separates with a red color or wine-tint.

The indications which may be deduced from these tests for coloring matter, are exceedingly unsatisfactory; the presence of biliary pigments alone being of real diagnostic value. It may generally be assumed, however, that a urine whose color is greatly intensified by the

addition of nitric or hydrochloric acid, is not normal, but the cause will usually be a matter of speculation, unless a quantitative analysis be made for the estimation of urea, chlorides, phosphates, and all of the constituents whose proportions may be indicative of alteration in the processes of disassimilation.

§ 138. *Blue and violet urine.*—Sometimes urine has a blue or violet color, which may appear either throughout the whole liquid, or only on its surface, or on the surface of the vessel containing it. This coloring matter is more commonly found in stale urine, and will usually be left on the filter-paper when the liquid is filtered. When such urine is agitated with ether or chloroform the coloring matter is taken up by the reagent, which separates with a rose, violet, or blue tint; if the urine be previously filtered, the blue coloring matter generally remains on the filter, so that the ether or chloroform assumes only a light rose-color.

The nature of the red coloring matter, which is usually present with the blue in these cases, is not known, for it only occurs in very minute quantities, its coloring properties being very intense.

The blue matter seems to be identical with indigotine, and to be formed from the indican naturally existing in the urine (see § 110). It is highly probable that the red coloring matter is also derived from indican, for the latter decomposes into indigotine and a red matter called indirubin, which has already been mentioned (§ 112).

CYSTINE.

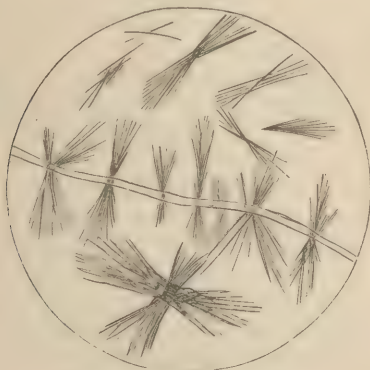
§ 139. Cystine is seldom found in the urine; when it exists there, it is generally deposited as sediment soon after the urine is voided, but occasionally it may remain dissolved for some time. Urine containing cystine is usually of a pale, sometimes a greenish color, and often possesses the odor of hydrogen sulphide. Urine having such an odor should be examined for cystine, and may lead to a diagnosis of cystic vesical calculus. Cystine is precipitated from urine on the addition of acetic acid, and may then be recognized by its crystalline form under

the microscope, and by its chemical tests (§ 74). Before applying the lead oxide and potassium hydrate test, any albumen present must be removed from the urine, for albuminoid matters contain sulphur, and would produce a black precipitate when boiled with an alkaline solution of lead oxide.

LEUCINE AND TYROSINE.

§ 140. These substances are seldom present in urine, but are almost invariably found in cases of acute atrophy of the liver, and sometimes in such abundance that a drop of the urine placed upon a microscope slide will solidify in a mass of crystals of leucine and tyrosine. They have also been found in the urine in fevers of a typhoid character. In such cases but little urea is present, albuminoid substances seeming to be eliminated principally in the form of leucine and tyrosine.

Fig. 27.



Tyrosine deposited from urine.

Sometimes the tyrosine separates as sediment; it is then recognized by its microscopic appearance (Fig. 27) and by the characters described in § 62.

To detect leucine and tyrosine dissolved in urine, the latter is freed from albumen, treated with basic lead acetate, the precipitate separated, and the liquid freed

from lead by means of hydrogen sulphide. The clear liquid, concentrated on a water-bath, gradually deposits tyrosine, which is purified by solution in boiling water, and recrystallization. The characters of tyrosine are described in § 62.

Leucine is obtained from the mother-liquor from which tyrosine has deposited; this is concentrated, and treated first with cold and then with boiling absolute alcohol, as long as the latter dissolves anything; the brown residue will still contain tyrosine. The alcoholic extracts are united, evaporated to a syrupy consistence, and allowed to crystallize. After several days a granular deposit forms, consisting of crystals of leucine mixed with fatty matters. These are collected, pressed between folds of filter-paper, dissolved in ammoniacal water, and basic lead acetate is added to the solution as long as it produces a precipitate. The latter is a compound of leucine and lead oxide; it is collected on a filter, washed with a little water, then suspended in water and decomposed by hydrogen sulphide. When sufficiently concentrated, the filtered liquid deposits crystals of leucine. These are examined according to § 61.

If the urine be stale, it will contain no leucine, that body being then converted into ammonium valerate.

BLOOD.

§ 141. Urine containing blood is dark in color, sometimes red, sometimes brown, and sometimes even blackish. Three cases may be presented: 1st, the blood may be present as such, and red corpuscles will then be found on a microscopic examination of the sediment; 2d, no corpuscles may be present, but the natural coloring matter of the blood, hemoglobin, can be detected; 3d, the decomposition may have gone so far that only hematin can be recognized. In the latter two cases, the aid of the spectroscope is required to clearly demonstrate the presence of the coloring matter of blood (§§ 87-88). In the first case, the microscope gives satisfactory evidence. All urine containing blood also contains albumen, and the latter may be detected as already indicated. The red

corpuseles retain their characteristic form for some time in acid urine, but, if the latter be alkaline, they are rapidly destroyed (see § 183).

AMMONIA.

§ 142. The presence of ammonium carbonate in urine is detected according to section 129. It must be borne in mind that stale urine will always contain more or less ammonium carbonate, formed by the decomposition of urea.

Rapid Qualitative Analysis of Urine.

§ 143. It is sometimes desirable to make a rapid chemical analysis of urine, such as may be practised at the bedside of a patient; in such a case, the physical characters are first observed; the color, transparence, odor, reaction, specific gravity; if any sediment be present, this must be subsequently examined microscopically. Portions of the urine are then tested:—

1. For albumen, by the nitric acid test.
2. For glucose; the specific gravity will generally have indicated whether it be important to make this test.
3. Biliary acids by Pettenkofer's test, and biliary pigments by Gmelin's test. Pettenkofer's test seldom succeeds when thus applied directly to the urine.

While such a brief examination can never be accepted as conclusive, yet it will frequently be of service, as it may show that the urine is normal, or that a more careful chemical examination is necessary.

Quantitative Analysis of Urine.

ESTIMATION OF NORMAL CONSTITUENTS.

§ 144. The quantitative analysis of urine is undertaken for the determination of the proportions of the various constituents, normal or abnormal, which may be present. The methods which are employed are both gravimetric and volumetric, and the latter are always

preferable when their results are equally trustworthy as those of the former; they are more rapid, and demand less expensive apparatus and less practice in manipulation.

It has already been mentioned that the composition of the urine varies considerably with the hours of the day at which it is voided. For this reason, a quantitative analysis should be made upon a portion of the mixed urine of the twenty-four hours; if this be impossible, the urine passed in the morning should be analyzed by preference. In any case, the results should be so calculated as to express the proportions of the various constituents eliminated in twenty-four hours; hence it is important that the daily volume of the excretion be known.

Should any sediment be present, it is separated by filtration, and the quantitative operations conducted upon the filtrate.

The following figures have been given as representing about the proportions of the various substances eliminated by the kidneys in twenty-four hours, and the centesimal composition of average normal urine.

	Quantity in grammes eliminated per day.	Composition of 1 kilo. of urine.
Water	1238.07	952.36
Urea	31.55	24.27
Uric acid	0.52	0.40
Hippuric acid	1.30	1.00
Creatinine	1.30	1.00
Xanthine	0.006	0.004
Pigment and ill-defined extractive matters	7.065	5.435
Sodium chloride	13.30	10.231
Alkaline sulphates	4.03	3.10
Phosphates calcium	0.408	0.314
magnesium	0.591	0.455
sodium	1.86	1.431
	<hr/> 1300.000	<hr/> 1000.000
Water	1238.07	952.36
Organic matter	41.74	32.11
Mineral matter	20.19	15.53
	<hr/> 1300.00	<hr/> 1000.00

ESTIMATION OF WATER AND OF FIXED MATTERS.

§ 145. Weigh 10 c.c. of the urine in a platinum capsule or porcelain crucible, the weight of which has already been determined, and evaporate to dryness in a hot-water oven at 100° , until the weight of the residue remains sensibly constant. As this residue is quite hygroscopic, the capsule should be covered during the weighing. The difference between the weight of the urine and that of the residue is the weight of water contained in 10 c.c. of the urine; the weight of the residue is the total fixed matter, organic and mineral. The residue is now incinerated at a red-heat over a Bunsen burner or an alcohol lamp. The residue consists of the fixed mineral matters; the difference between the weight of this latter and that of the total fixed matter, is the weight of the solid organic matter contained in the 10 c.c.

This method yields only approximate results; in certain cases it may be accurate, but, since few specimens of urine can be evaporated without decomposing a considerable proportion of the urea present, it can readily be understood that the method has but little real value. However, it may be used for the estimation of the inorganic constituents; should the urine be poor in solid matters, a fact at once indicated by a low specific gravity, it may be necessary to evaporate 20, 50, or even 100 c.c., instead of 10 c.c., which is generally sufficient for healthy urine.

Magnier de la Source recommends that about 2 c.c. of urine be accurately weighed between two watch-glasses, and evaporated at the ordinary temperature in a vacuum. In twenty-four hours the dry residue may be weighed, and the quantities of water and of fixed matter can be calculated. This method appears to give very accurate results, the figures given by the author being quite satisfactory.

Neubauer proposed a method for the calculation of the fixed matter from the specific gravity, and the approximation is very close in the case of normal urine; for pathological urine, however, its results are not so trustworthy. It consists in multiplying the last two figures

of the observed density by the constant factor 2.33; thus were the specific gravity 1024 ($24 \times 2.33 = 55.92$), 1000 parts of urine would contain 55.92 parts of solid constituents. This approximate calculation is sufficiently accurate for the purposes of physicians.

The presence of the alkaline and earthy metals, and of the mineral acids with which they are combined, may be detected in the residue of the last incineration by the usual chemical tests.

Normal urine contains from 40 to 65 grammes of solid matter per litre, of which between one-fifth and one-third is composed of anhydrous mineral salts.

I.—Organic Constituents.

UREA.

§ 146. A healthy adult man excretes from 15 to 30 grammes of urea per day; women eliminate somewhat less.

Numerous methods have been devised for estimating the proportion of urea contained in urine; of these, two are quite trustworthy, while their application is easy, and either of them may be employed.

§ 147. LIEBIG'S METHOD depends upon the precipitation of the urea by a solution of mercuric nitrate of known strength (see § 39).

The reagents and apparatus required are—

1st. A titrated solution of mercuric nitrate, of which 1 c.c. corresponds to exactly 10 milligrammes of urea.

2d. A baryta solution, made by mixing two volumes of cold saturated baryta-water with one volume of a cold saturated solution of barium nitrate.

3d. A rather strong solution of sodium carbonate.

4th. A burette of 50 c.c. capacity.

5th. A volume pipette of 15 c.c. capacity.

Preparation of the solution of mercuric nitrate.—a.—4 grammes of pure urea, dried over sulphuric acid in a vacuum, are dissolved in distilled water, and the solution is diluted to exactly 200 c.c. 10 c.c. of this solution contain 200 milligrammes of urea.

b.—96.855 grammes of perfectly dry and chemically pure mercuric chloride are dissolved in distilled water, and a dilute solution of sodium hydrate is added as long as it forms a precipitate. The latter is allowed to deposit completely, the clear liquid is decanted, and the precipitate is washed with distilled water, first by decantation, then upon a filter. After the washing is terminated, the filter is pierced, and all of the precipitate is carefully washed into a flask or beaker; when the mercuric oxide has subsided, the water is poured off, and the mercuric oxide is dissolved in a sufficient quantity of nitric acid containing no nitrous acid. The solution is diluted to nearly one litre, and its exact volume is measured.

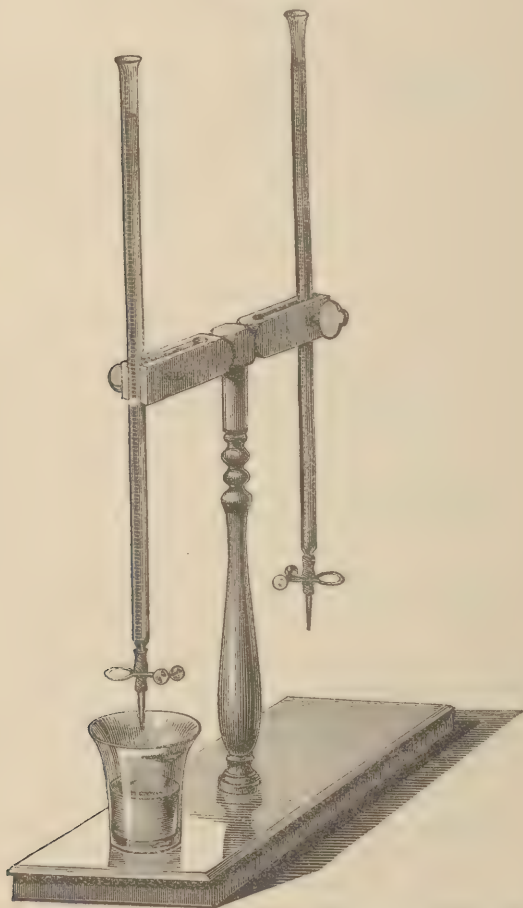
10 c.c. of the normal solution of urea (*a*) are measured into a beaker, and into this liquid the solution of mercuric nitrate is allowed to flow from a burette (Fig. 28), until a drop of the mixture, removed by the aid of a glass-rod, produces a distinct yellow color with a drop of sodium carbonate solution. The number of cubic centimetres of the mercury solution required to effect this reaction is read off exactly; if the solution were exactly titrated, just 20 c.c. would have been employed, but as it is thus far only approximate, the number will be somewhat less than 20 c.c. The mercury solution is therefore diluted with exactly sufficient distilled water to make 20 c.c. equivalent to 10 c.c. of the urea solution.

For example, if 19.25 c.c. of the approximate mercury solution be required to precipitate 10 c.c. of the urea solution, 0.75 c.c. of distilled water should be added for every 19.25 c.c. of the original solution, or 7.5 c.c. of water for every 192.5 c.c. of the mercury solution. If then the approximate solution measure 962.5 c.c., it must be diluted with 37.5 c.c. of water. If only 18 c.c. of the mercury solution were required, for every 180 c.c., 20 c.c. of water must be added.

After exactly diluting the mercury solution, its strength is confirmed by a new trial on 10 c.c. of the urea solution. Just 20 c.c. should be required to produce the final reaction with sodium carbonate. The titrated solution is kept in well-stoppered bottles.

Practice of the analysis.—The analysis by Liebig's method requires that the phosphates shall be first com-

Fig. 28.



pletely precipitated from the urine ; this is accomplished by the baryta solution.

A certain volume of the urine, say 40 c.c., is exactly measured, and mixed with half its volume, that would be

20 c.c., of the baryta solution. The liquid is well mixed by stirring with a glass rod, and the precipitate is separated by filtration. 15 c.c. of the filtrate, which would correspond to 10 c.c. of the original urine, are measured into a beaker, and the normal solution of mercuric nitrate is allowed to flow in, drop by drop, from the burette, until a drop of the mixture, removed by the aid of a glass rod, produces a distinct yellow color with a drop of the sodium carbonate solution. This color is best seen upon a black surface, such as is obtained by placing a glass plate or watch-glass upon black glazed paper.

The volume of the mercury solution used is then read off in cubic centimetres, and this number multiplied by ten gives the number of milligrammes of urea contained in 10 c.c. of the urine analyzed.

Corrections.—This method only gives exact results in solutions containing 2 per cent. of urea; therefore, if more than 30 c.c. of the standard solution be required to precipitate the urea from the 15 c.c. of diluted urine employed, the mixture will contain more than two per cent. of urea, and it is then necessary, before the final trial with sodium carbonate, to add to the mixture a volume of distilled water equal to half the number of cubic centimetres of mercury solution used in excess of 30 c.c. For example, if 50 c.c. of the standard solution have been employed ($50 - 30 = 20$), 10 c.c. of distilled water are added to the mixture. Otherwise the amount of urea indicated by the analysis will be less than that really present.

If, on the contrary, the urine contain less than two per cent. of urea, the proportion of the latter will appear greater than it really is; this error is corrected by subtracting from the number of cubic centimetres of the mercury solution used one-tenth of a cubic centimetre for every 5 c.c. less than 30; for example, if only 20 c.c. be required to produce the yellow color with sodium carbonate, two-tenths of a c.c. are subtracted, and $19.8 \text{ c.c.} \times 10$ will express in milligrammes the quantity of urea present.

If a large proportion of sodium chloride be present in the urine, the results by Liebig's method will be too

high, since mercuric chloride would then be formed, and this does not precipitate urea. If the proportion of sodium chloride be small, the error may be neglected, but, if it amount to 1 or 1.5 per cent., 2 c.c. are subtracted from the volume of mercury solution used to precipitate 10 c.c. of urine. The result is then approximately correct; but if an exact estimation of urea be required, the chlorides must first be precipitated by silver nitrate, as follows:—

The 15 c.c. of prepared urine are exactly neutralized with nitric acid, and a drop or two of a saturated solution of neutral potassium chromate is added. A solution of silver nitrate is then allowed to flow from a burette into the mixture, until the white precipitate at first formed assumes a distinct orange color. The titration by the solution of mercuric nitrate is then conducted in the liquid, as already directed, without removing the precipitated silver chloride. Of course, the increased volume of the original 15 c.c., due to the addition of the silver solution, must be borne in mind in making the first correction mentioned.

§ 148. YVON'S METHOD.—This method depends upon the decomposition of the urea by sodium hypobromite, and the determination of the quantity of nitrogen set free (see § 38). Since the estimation of the nitrogen requires corrections for temperature and pressure, the process has been slightly modified, and rendered more rapid, by a comparison of the volume of nitrogen disengaged from a known quantity of the urine with that eliminated from a known quantity of urea at the time of the experiment.

The apparatus and reagents required are—

1st. A ureometer (Fig. 29). This is a glass tube, about 40 centimetres long, divided into two unequal compartments by a glass stopcock. Each section of the tube is graduated into cubic centimetres and tenths of cubic centimetres, numbered from the stopcock.

2d. A solution of urea containing 2 per cent. of urea, made by dissolving 4 grammes of perfectly dry pure urea in 200 c.c. of distilled water.

3d. An alkaline solution of sodium hypobromite, pre-

pared by dissolving 17 grammes of sodium hydrate and 5 grammes of bromine in 133 c.c. of distilled water.

A deep vessel, preferably of iron, nearly filled with mercury, and a tall jar of water into which the ureometer may be plunged up to the stopcock, will also be necessary; the apparatus may be simplified and the process shortened by fitting a rubber cork and caoutchouc tube to the lower end of the ureometer, the extremity of the caoutchouc tube being adapted to any suitable glass mercury reservoir which may be raised and lowered on a support, as necessary. The ureometer is also clamped to the support in a vertical position. The operation will be described, supposing this latter arrangement to be adopted; the manipulation by the use of the deep mercury trough will then be self-evident.

The analysis is conducted upon 1 c.c. of urine.

The stopcock of the ureometer being open, the mercury reservoir is raised until the mercury rises in the ureometer and exactly fills the lower portion. The stopcock is then closed, and the mercury reservoir is fixed at this level.

10 c.c. of the urine are diluted with distilled water to exactly 50 c.c.

5 c.c. of the normal solution of urea are poured into the upper compartment of the ureometer, the mercury reservoir is lowered, and, on opening the stopcock, the urea solution will flow into the lower compartment. Care must be taken that no air enters, and, as soon as all of the solution has passed the stopcock, the latter is closed. The upper compartment is then washed out with two or three c.c. of rather dilute sodium hydrate solution, which is also allowed to flow into the lower part of the tube. The upper compartment is now completely filled with the hypobromite solution, and the latter is rapidly passed into the urine by opening the stopcock and depressing the mercury reservoir. If any air-bubbles be allowed

Fig. 29.



to enter, it will be necessary to recommence the whole operation. By alternately raising and lowering the mercury reservoir, the solutions become thoroughly mixed, and nitrogen collects in the tube. The 5 c.c. of urea solution used contained one centigramme of urea; at 0° , and under a pressure of 760 millimetres, this would yield 3.5 c.c. of nitrogen. However, no attention is paid to the temperature and pressure, but when the evolution of gas has ceased, the volume of nitrogen is read off, the mercury being brought to the same level in the ureometer and in the reservoir. •

The liquid is then removed from the ureometer by opening the stopcock, and raising the mercury reservoir; and the tube is thoroughly washed out with distilled water. After some practice in the manipulation, the washing may be done quite rapidly.

The upper compartment is carefully dried by filter paper, and 5 c.c. of the diluted urine, containing 1 c.c. of urine, are introduced, and operated upon precisely as has been described for the urea solution. Then—

Vol. of N yielded by 1 centigramme urea	: 1 ::	Vol. of N yielded by 1 c.c. of urine	: No. of centigrammes of urea in 1 c.c. of urine.
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Example.— 5 c.c. of the normal solution of urea have produced 3.9 c.c., or 39 divisions, of nitrogen. At the same time, 5 c.c. of the diluted urine, containing 1 c.c. of urine, have yielded 5.8 c.c., or 58 divisions, of nitrogen. Hence 1 c.c. of the urine contains $\frac{58}{39}$ centigrammes of urea; or 1000 c.c. of urine contain 14.87 grammes of urea. If it be desired to estimate the amount of urea in 1 kilogramme of urine, the quantity contained in one litre is multiplied by 1000 and divided by the specific gravity: thus if the density of the urine in the example already given be 1018, $\frac{14.87 \times 1000}{1018} =$

14.607, the quantity of urea in 1 kilogramme of urine.

Precautions.—The stopcock of the ureometer must be frequently greased, otherwise it will allow the entrance of air. The sodium hypobromite solution does not keep well, especially in warm weather, and should be

frequently renewed. If the proportion of urea be extremely small (indicated by the specific gravity), the urine is not diluted, and two, three, or even five c. c. are employed. If, on the contrary, the proportion of urea be very great, it may be necessary to limit the analysis to half a cubic centimetre; in this case 5 c. c. are diluted to 100 c. c. and 5 c. c. of the diluted urine are employed as before.

This method is quite accurate, and sufficiently rapid: the entire operation may be concluded in fifteen or twenty minutes. The ureometer must be thoroughly washed with water or dilute hydrochloric acid, after each operation.

§ 149. ESBACH'S METHOD.—This method also depends upon the decomposition of urea by sodium hypobromite, but the apparatus is much simpler, and the operation more rapid than in Yvon's process. The only apparatus required is a stout glass tube about 40 centimetres long, closed at one end, and containing about 28 c. c. It is graduated, beginning at the closed end, in cubic centimetres, and tenths of cubic centimetres.

A normal solution of urea, and a sodium hypobromite solution are required. These are prepared precisely as in Yvon's process. 6 c. c. of the sodium hypobromite solution are poured into the tube, and about 8 c. c. of water are carefully added, so that the two liquids may not mix. The volume of liquid is then read, say 14.7 c. c. By the aid of a pipette, exactly one cubic centimetre of the urine to be examined is introduced, being allowed to flow down the sides of the tube, and the latter is immediately closed by the thumb protected by a piece of sheet caoutchouc, or by a caoutchouc cork, and vigorously agitated. The volume of liquid in the tube is equal to the initial volume plus 1 c. c., say 15.7 c. c. When no more gas is disengaged, the open extremity of the tube is plunged into water, and the thumb removed, or the caoutchouc stopper withdrawn. A quantity of liquid is then expelled equal in volume to that of the nitrogen set free. The tube is then depressed in the water until the level of the interior liquid coincides with that of the water in the exterior vessel; the extremity is again

closed by the thumb or a caoutchouc cork, and the instrument is withdrawn, held vertically with the closed extremity down, and the volume of liquid read off. The difference between this reading and the total volume of liquid used, gives the volume of nitrogen formed. If, for example, 9.3 c. c. of liquid remain, $15.7 - 9.3 = 6.4$, the nitrogen eliminated by 1 c. c. of urine.

The operation is repeated in the same manner, the 1 c. c. of urine being replaced by 5 c. c. of the normal urea solution; the volume of nitrogen generated is determined by the volume of liquid it expels, and the subsequent calculations are made precisely as in Yvon's process.

This method is very rapid, and sufficiently accurate. If the ureometer be closed by the thumb, the latter should be blown upon horizontally before removing it for the final reading, in order that adhering water may not run into the tube. If a caoutchouc cork be employed, this must always be inserted to the same point.

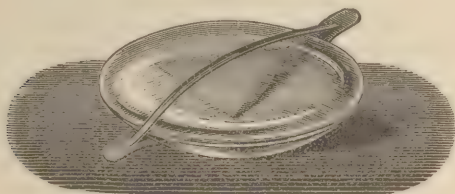
URIC ACID.

§ 150. The quantity of uric acid excreted, is as variable in health as in disease, ranging from 0.3 to 0.8 gramme per day. The average amount is about 5 decigrammes; it is much increased by an animal diet, and correspondingly diminished when but little nitrogenized food is taken.

The proportion of uric acid present in urine can only be determined, with any degree of accuracy, by the balance. If albumen be present, the urine must first be boiled, rendered freely acid with acetic acid, and filtered boiling. In any case, it must be filtered to separate mucus and other suspended matters. From 200 to 400 c. c. of the clear, filtered urine, are then mixed with three or four per cent. of concentrated hydrochloric acid, and the mixture is allowed to stand in a cool place for twenty-four or forty-eight hours. The uric acid then separates in the crystalline form. The operation should be performed in a vessel which may be easily cleaned by the fingers, since the crystals sometimes obstinately ad-

here to the sides of the vessel. The precipitated acid is collected on a tared filter, the glass rinsed with 5 or 10 c. c. of distilled water to wash the uric acid into the filter, and any crystals still remaining are removed by the finger and washed into the filter with a little alcohol. The glass should be washed with alcohol until no crystals can be seen by the aid of a magnifying glass. All of the washings are passed through the filter. The latter, with its contents, is dried at 100° , and weighed between two watch glasses. (Fig. 30.)

Fig. 30.



This method is not absolutely accurate, nor is any other for the estimation of uric acid, for a small quantity of the latter is lost by its slight solubility in acid urine, and with the water used for washing. The quantity of the latter should be limited, if possible, to about 10 c. c. If, however, more than 30 c. c. be employed, .045 milligramme are added to the weight of uric acid found, for every cubic centimetre of water in excess of 30. (Neubauer.) If, for example 55 c. c. of wash water be used, $55 - 30 = 25 \times .045 = 1.125$ milligrammes must be added to the amount of uric acid found by the balance.

On the contrary, the precipitated uric acid always retains some coloring matter, and the latter compensates, in a measure, for the necessary loss, unless, as has been said, the precipitate be washed with more than 30 c. c. of water.

HIPPURIC ACID.

§ 151. The amount of hippuric acid eliminated by the kidneys, seldom exceeds 3 or 4 decigrammes in twenty-four hours, unless certain vegetable medicaments or vege-

table aliments be ingested. Consequently, large quantities of urine are required for the estimation of this acid. It may be separated by the following process, proposed by Meissner. One litre of fresh urine is completely precipitated by baryta water, and the excess of baryta is thrown down by dilute sulphuric acid, carefully avoiding an excess. The filtered liquid is exactly neutralized with hydrochloric acid, and evaporated to a syrupy consistence on a water-bath. The residue is agitated with 150 or 200 c. c. of absolute alcohol, which dissolves the hippurates, and leaves the chlorides, etc., undissolved. The clear alcoholic solution is decanted, evaporated to a syrupy consistence, and rendered strongly acid by hydrochloric acid. It is then agitated with 100 or 150 c. c. of alcoholic ether, which dissolves the hippuric acid, and leaves it in an impure state on evaporation. It is purified by boiling with a small quantity of milk of lime, decolorizing by animal charcoal, filtering the solution while boiling, and adding hydrochloric acid to the still hot filtrate. The hippuric acid deposits in needles as the liquid cools. It is then collected, dried, and weighed.

As hippuric acid is not very soluble in ether, a sufficiently large quantity of alcoholic ether must be employed to effect its solution after it is first deposited.

CREATININE.

§ 152. A healthy adult excretes from 6 to 16 decigrammes of creatinine per day ; the quantity is diminished by a vegetable diet, and correspondingly increased by an exclusively animal alimentation.

200 or 300 c. c. of the urine, free from albumen, are rendered alkaline by milk of lime, and treated with calcium chloride as long as the latter forms a precipitate. After one or two hours the liquid is filtered, the precipitate washed, and the filtrate, together with the washings, rapidly evaporated to a syrupy consistence on a water-bath. The still warm liquid is agitated with 40 or 45 c. c. of 95 per cent. alcohol, and the mixture is poured into a glass, and allowed to stand in a cool place for six or eight hours. The liquid is then filtered through a very

small filter, and the precipitate is washed with a little alcohol, which is added to the filtrate. If the total volume of liquid exceed 60 c. c., it is reduced to 50 or 60 c. c. by evaporation on a water-bath, and when quite cold, half a cubic centimetre of a concentrated and neutral alcoholic solution of zinc chloride is added, and the mixture briskly agitated. It is then allowed to stand in a cool place for two or three days, in which time the double compound of creatinine and zinc chloride separates in small crystals. The latter are collected on a small, tared filter, allowed to drain, and washed with a little alcohol, until the latter passes through colorless, and yields no precipitate with silver nitrate. The filter with its precipitate is then dried at 100° , and weighed. (Neubauer.

100 parts of the double chloride of zinc and creatinine correspond to 62.44 parts of creatinine.

II. Mineral Constituents.

SODIUM CHLORIDE.

§ 153. The quantity of sodium chloride contained in the urine is extremely variable, depending, as is evident, on the state of the health and the nature and quantity of food taken. It ordinarily forms about two-thirds of the total fixed salts, or ash, and it seems that the daily elimination may be comprised between five and twelve grammes. It is usual to determine the quantity of chlorine contained in the urine, and to estimate the entire amount as sodium chloride; the daily elimination of chlorine would then be from 3 to 7 grammes, representing from 5 to 11 grammes of sodium chloride; the average excretion is nearer the latter figure.

Solutions of chlorides and of phosphates are both precipitated by silver nitrate, but if a drop or two of potassium chromate solution be introduced into a liquid containing chlorides and phosphates, and silver nitrate then be added, it is found that red silver chromate is formed as soon as the chlorides are entirely precipitated, while the phosphates remain in solution until all of the potassium chromate is decomposed.

The chlorine contained in urine may hence be volumetrically estimated by a standard solution of silver nitrate. This is prepared by dissolving 29.065 grammes of pure, fused silver nitrate in distilled water, and diluting the solution to exactly one litre. One cubic centimetre of this solution corresponds to 10 milligrammes of sodium chloride, or to 6.068 milligrammes of chlorine.

10 c.c. of the urine are mixed with 2 grammes of pure potassium nitrate, and cautiously evaporated to dryness in a platinum or porcelain capsule: it is better that this evaporation should be conducted on a water-bath. The residue is then carefully heated until it fuses, and all traces of carbonaceous matter have disappeared. The cold mass is dissolved in about 30 c.c. of distilled water, and the liquid is transferred to a beaker glass, and slightly acidified by a drop or two of dilute nitric acid: after which the excess of acid is saturated, and the solution rendered neutral, by the addition of a little pure calcium carbonate. A few drops of a saturated solution of potassium neutral chromate are added, and the silver nitrate is dropped into the mixture from a burette filled to the zero division, until the precipitate formed assumes a distinct orange color, which is persistent, and does not disappear on agitation. The number of cubic centimetres of the silver solution used, multiplied by 10, expresses in milligrammes the quantity of sodium chloride contained in 10 c.c. of the urine analyzed. Suppose 9.8 c.c. of the silver nitrate solution be required to produce the persistent tint of silver chromate: then 10 c.c. of the urine contain $9.8 \times 10 = 98$ milligrammes of sodium chloride, and 1000 c.c. of the urine contain 9.8 grammes of sodium chloride, or $9.8 \times 6.068 \times 100 = 5.946$ grammes of chlorine.

During febrile conditions of the system, the proportion of sodium chloride in the urine is much diminished, and gradually becomes normal with returning health.

PHOSPHORIC ACID, PHOSPHATES.

§ 154. Phosphoric acid exists in the urine as sodium acid phosphate, magnesium acid phosphate, and calcium

acid phosphate. The quantities of these salts eliminated per day, varies considerably, their sum generally corresponding to between one and two and a half grammes of phosphoric anhydride. The following figures have been given as representing the daily urinary excretion of the phosphates, expressed in phosphoric anhydride:—

As sodium acid phosphate . . .	1.5 grammes.
As magnesium acid phosphate . . .	0.6 “
As calcium acid phosphate . . .	0.2 “

The sodium salt is soluble, but the solubility of the others is due to the acid character of the urine, and when the latter becomes alkaline, they are deposited.

It is usual to estimate separately the total phosphoric acid, and that existing as earthy phosphates, that present as alkaline phosphates being estimated by difference.

The most convenient method for the estimation of phosphoric acid is volumetrically, by precipitation as uranium phosphate, a standard solution of uranium acetate being used.

In addition to a burette and volume pipette, the following solutions will be required.

1st. A solution of sodium acetate, made by dissolving 100 grammes of sodium acetate, and 100 c.c. of concentrated acetic acid in distilled water, and diluting to one litre.

2d. A solution of sodium phosphate, of which 50 c.c. shall correspond to exactly 0.1 gr. of phosphoric anhydride. This is made by dissolving 10.085 gr. of pure crystallized sodium phosphate ($\text{Na}^2\text{HPO}_4 + 12\text{H}^2\text{O}$) in 1000 c.c. of distilled water.

3d. A solution of uranium acetate, made by dissolving about 35 grammes of uranium acetate in distilled water, adding about 5 c.c. of strong acetic acid, and diluting the whole to one litre. As this solution generally deposits a precipitate, it should be allowed to stand some time before being titrated.

50 c.c. of the sodium phosphate solution are measured into a beaker, and 5 c.c. of the sodium acetate solution added. The mixture is then stirred and heated to 90 or 100°, preferably on a water-bath. A burette is filled

with the uranium acetate solution, and the latter is allowed to flow gradually into the hot sodium phosphate, with continual stirring, until a drop of the liquid, removed by the aid of a glass rod, produces a reddish-brown color when placed on some powdered potassium ferrocyanide, or in a drop of a strong solution of the latter salt. The number of cubic centimetres of the uranium solution used is now read off, and its exact strength is calculated. Suppose 20.5 c.c. of uranium solution to be required to produce the final reaction with potassium ferrocyanide. Since the 50 c.c. of sodium phosphate solution corresponds to 0.1 gr. of phosphoric anhydride, one cubic centimetre of the uranium solution will be equivalent to $\frac{.1}{20.5} = .00487$ gr. of phosphoric

anhydride. The uranium solution is so prepared that each cubic centimetre shall precipitate 5 milligrammes of phosphoric acid; if its strength be found greater than this, the calculated amount of distilled water is added, but if more than 20 c.c. be required for 50 c.c. of the normal solution of sodium phosphate, the liquid may be used as it is, its exact strength being found as above.

Analytical process.—The analysis must be performed precisely as the titration of the uranium solution. 50 c.c. of the filtered urine are mixed with 5 c.c. of the sodium acetate solution, heated nearly to boiling, and the uranium acetate is added, drop by drop, from a burette, until a drop of the liquid produces a reddish-brown color with potassium ferrocyanide. The urine must be kept hot, and the termination of the reaction is best detected by distributing twenty or thirty drops of potassium ferrocyanide solution on a porcelain plate, before beginning the analysis, and touching these successively with drops removed from the urine under examination, after the addition of every $\frac{1}{2}$ c.c. of the uranium solution. The number of c.c. of the latter solution used, multiplied by the equivalence of 1 c.c. in phosphoric anhydride, as already determined, gives the amount of phosphoric anhydride in 50 c.c. of the urine.

Estimation of the phosphoric acid present as earthy phosphates.—200 c.c. of the urine are rendered strongly

alkaline by ammonia, and allowed to stand twelve hours. The precipitate of earthy phosphates is then collected on a small filter, washed with ammonia water, and transferred to a beaker by piercing the filter and washing the precipitate through the opening by the aid of a wash-bottle. It is then dissolved in as small a quantity of acetic acid as possible, and, after the addition of 5 c.c. of the sodium acetate solution, the liquid is diluted to 50 c.c. with distilled water, and titrated with the uranium solution as before. The result of the analysis gives the amount of phosphoric anhydride corresponding to the earthy phosphates in 200 c.c. of the urine.

The proportion of phosphoric anhydride existing as alkaline phosphates is estimated by deducting that determined as earthy phosphates from the total amount found in the urine.

SULPHURIC ACID.

§ 155. An adult man normally eliminates alkaline sulphates corresponding to between two and three grammes of sulphuric acid daily. If it be desired to estimate accurately the quantity excreted, about 100 c.c. of the urine should be evaporated to dryness, after the addition of about 20 grammes of potassium nitrate, and the residue should be heated until all carbonaceous matter has disappeared. The mass is then dissolved in dilute hydrochloric acid, and the solution is precipitated boiling with barium chloride. The precipitated barium sulphate is collected on a filter, washed with hot water, dried, and weighed. 100 parts of barium sulphate correspond to 42.06 parts of sulphuric acid.

It is seldom required to estimate sulphuric acid in the foregoing manner; Vogel places the average daily excretion at about 2 grammes, and proposed the following approximate method for determining whether more or less than that quantity is eliminated. It is of course necessary, as in all other urinary estimations, that the daily volume of urine shall be known; if this amount to 1200 cubic centimetres, and the normal excretion of sulphuric acid be 2 grammes, 60 c.c. of urine should contain

0.1 gramme of the acid. In such a case, 60 c. c. of the urine are acidified with hydrochloric acid, and a solution of barium chloride containing exactly sufficient of the salt to precipitate 0.05 gramme of sulphuric acid, is added. The mixture is then filtered, and a drop of barium chloride is added to the filtrate; if no turbidity be produced, the daily excretion of sulphuric acid does not exceed one gramme; but if a precipitate form, a quantity of barium chloride equal to the first is added, the mixture is filtered, and the new filtrate is tested with barium chloride. If a precipitate be again formed, more than two grammes of sulphuric acid are eliminated in twenty-four hours. 10 c. c. of a solution made by dissolving 8.76 grammes of pure, dry barium chloride in one litre of water, will exactly precipitate 5 centigrammes of sulphuric acid.

Estimation of Abnormal Constituents.

ALBUMEN.

§ 156. The proportion of albumen present in urine can only be accurately determined by coagulation, and drying and weighing the precipitate. A convenient method by which the coagulation may be accomplished has been described in section 84. The chief difficulty experienced when the coagulation is effected by heat, is caused by the obstinacy with which the coagulum adheres together and resists washing. This may be obviated, in a measure, by operating as follows: 20, 50, or 100 c. c. of the filtered urine, according to the proportion of albumen present, is gently heated, and as soon as it begins to appear clouded, a drop or two of acetic acid is added. The albumen then precipitates in large flakes; when the coagulation appears complete, the coagulum is collected upon a filter which has been dried at 110° and weighed, and is washed, first with hot water, then with alcohol. After sufficient draining, the filter and contents are dried at 100° in an air oven, until no further diminution of weight is perceptible. If a very exact estimation be required, the weight of the

filter-ash should be known, and after the weight of the dried albumen has been found, the filter and contents should be thoroughly incinerated in a crucible, and the weight of the remaining mineral salts deducted from that of the precipitated and dried albumen.

As has already been indicated, any albumen present must be removed from the urine before quantitatively estimating urea, uric acid, sodium chloride, or phosphoric acid. This may be accomplished by heating the urine in a long-necked flask, so that it may not become more concentrated by evaporation, and performing the analytical operations upon a measured volume of the filtered liquid.

GLUCOSE.

§ 157. The most trustworthy process for the estimation of glucose depends upon the reduction of a cupric solution. As has already been mentioned (§ 22), when glucose is boiled with an alkaline solution of cupric oxide, the latter is reduced to cuprous oxide, and it has been found that one molecule of glucose is capable of reducing five molecules of cupric oxide, that is, 180 parts of glucose reduce the cupric oxide contained in 1247.5 parts of crystallized cupric sulphate.

The cupric solution used is that proposed by Fehling, and is made as directed in section 33. Each c. c. contains 34.64 milligrammes of cupric sulphate, and is exactly reduced by 5 milligrammes of glucose. This solution is fit for quantitative analysis only when it deposits no precipitate after being boiled and allowed to stand about half an hour.

When the specific gravity of the urine, and a preliminary qualitative examination, have shown that a large proportion of glucose is present, the urine must be diluted with ten or twenty times its volume of water, and it is only when it contains but a very small quantity of sugar that the undiluted urine can be used for the analysis.

10 c. c. of the urine are therefore diluted to 100 c. c. with distilled water, and another portion of 10 c. c. is diluted to 200 c. c.

Exactly 10 c. c. of Fehling's solution are measured into a small flask or capsule, and diluted with 30 or 40 c. c. of water; the liquid is heated by the aid of a lamp, and as soon as it begins to boil, the urine diluted to ten times its volume is allowed to flow in from a burette. Towards the close of the experiment, the urine is added drop by drop, until the liquid above the precipitated cuprous oxide has entirely lost its blue color; during the whole operation, the mixture must be kept boiling. The analysis is then terminated; its accuracy may be tested by filtering a small part of the liquid from the precipitate, and dividing the filtrate into two portions, one of which is tested by hydrogen sulphide, or by potassium ferrocyanide, while the other is boiled with a little more Fehling's solution. If a precipitate be formed in either of the first two cases, the reduction is not complete, and more urine must be added; if the drop of Fehling's solution added to the second portion be reduced, too much urine has been added, and the whole operation must be repeated.

If only a few drops of the urine diluted to ten times its volume suffice to reduce the 10 c. c. of Fehling's solution, the experiment must be repeated with the urine diluted to twenty volumes.

Example.—If 11.5 c. c. of urine diluted to ten volumes be required to reduce 10 c. c. of Fehling's solution, these 11.5 c. c. must have contained 50 milligrammes of glucose; hence 10 c. c. of the undiluted urine contain

$$\frac{50 \times 100}{11.5} = 434.7 \text{ milligrammes, or } 1000 \text{ c. c. contain } 43.47$$

grammes. The result, in grammes per 1000 c. c., is therefore obtained by dividing 5×100 by the number of cubic centimetres used, when the urine is diluted to ten volumes, or by dividing 5×200 by the number of c. c. employed when the urine is diluted to 20 volumes.

When urine contains both glucose and albumen, the latter must be removed before making an analysis for the glucose. The coagulation may be effected as directed in the preceding section.

AMMONIA.

§ 158. The most convenient method for the estimation of ammonia in urine is that of Schlösing and Neubauer. It depends upon the facts that all the ammonia present in organic matters is eliminated when they are mixed with milk of lime, and that all of the ammonia in a closed space is readily absorbed by sulphuric acid.

The following solutions are needed:—

1. A decinormal solution of sulphuric acid: 1 c.c. = .0049 gr. H^2SO^4 .

2. A decinormal solution of sodium hydrate: 1 c.c. = .0040 gr. NaOH . 1 c.c. of the former solution is exactly neutralized by 1 c.c. of the latter. (See Appendix.)

3. Solution of litmus.

10 c.c. of the decinormal acid are placed in a small dish, over which is supported a capsule containing 20 c.c. of the urine. The whole is placed on a piece of plate-glass, about 10 c.c. of milk of lime are added to the 10 c.c. of urine, and the vessels are immediately covered with a bell-jar of which the edges are well greased. The apparatus is allowed to stand for about forty-eight hours, after which the sulphuric acid is titrated with the decinormal sodium hydrate. Had no ammonia been disengaged from the urine and absorbed by the acid, exactly 10 c.c. of the sodium hydrate solution would be required to neutralize the acid; every cubic centimetre less than 10, corresponds to 0.0017 gr. of ammonia.

This method is not quite accurate, the amount of ammonia found always being too great, for by the action of calcium hydrate a small quantity of urea is decomposed, disengaging ammonia.

Urinary Sediments.

§ 159. A few hours after emission, perfectly normal urine deposits a light cloud, composed of the débris of epithelial cells from the mucous membrane of the urinary tract, and no further change takes place until decomposition sets in. However, from various causes, a urine

may be turbid when voided; or it may be clear when passed, and deposit an abundant sediment on cooling. Urine which is acid does not often yield any deposit until it is cold, the sediment in this case consisting of urates, free uric acid, mucus, calcium oxalate or phosphate, and sometimes of hippuric acid or xanthine. On the contrary, alkaline urines are usually turbid when voided, and may hold in suspension pus, blood, large quantities of mucus, etc., and yield abundant sediments of earthy phosphates, ammonio-magnesium phosphate, and rarely earthy carbonates.

The urine is allowed to stand in a conical glass until the sediment has completely subsided, and as much as possible of the clear liquid is removed by decantation. The separation of the sediment may sometimes be hastened by the addition of a few drops of acetic acid, but it must be remembered that the latter reagent will dissolve certain deposits. A drop of the liquid remaining with the sediment is transferred to a glass slide by the aid of a pipette, and, after being covered with a piece of thin glass, is subjected to microscopic examination.

The sediment may be (1) crystallized, (2) amorphous, (3) organized, that is, containing cells, or (4) two or all of these forms may exist together.

The following table, taken from Marais' *Essai pratique des urines et des calculs urinaires*, together with the illustrations referred to, will aid in the identification of the sediment.

Microscopic form.	Characteristic reactions.	Substance.
<i>a. — Crystalline sediments.</i>		
Very large crystals, generally isolated, transparent, and having sharply defined angles: typical form that of a coffin-lid.	Soluble in acetic acid; not changed by potassium hydrate.	Ammonio magnesium phosphate (Figs. 31 and 32). Uric acid (Fig. 33).
Large crystals, generally grouped together, having a yellow or brown color, often having a cracked appearance, and dark outlines.	Insoluble in acetic acid: soluble in potassium hydrate.	
Small, transparent, isolated crystals, very refracting, sharp angles, octahedral form, often resembling the reversed side of an envelop.	Insoluble in acetic acid and in potassium hydrate.	Calcium oxalate (Figs. 34 and 35).
<i>Rare crystalline sediments.</i>		
Grayish sediment generally in alkaline urine. Colorless, hexagonal plates or in six-sided prisms.	Soluble in ammonia, from which they crystallize on evaporation; soluble also in sodium carbonate; precipitated from these solutions by acetic acid.	Cystine (Fig. 36).
Ill-defined crystals, having the form of a whetstone (quite rare).	Soluble in acids and alkalis. Its solution in nitric acid becomes yellow when evaporated; the residue is colored orange-yellow by potassium hydrate, and assumes a violet-red tint when heated.	
Four-sided rhombic prisms or needles, often mixed with crystals of uric acid and grouped with them. The urine is also very acid.	Soluble in potassium hydrate; insoluble in acetic acid; soluble in alcoholic ether.	Xanthine or hypoxanthine (see §§ 52 and 53).
Fine needles, grouped in tufts or stars.	Soluble in potassium hydrate and ammonia; reprecipitated by acetic acid (see § 149).	
<i>b. — Amorphous sediments.</i>		Tyrosine (Fig. 27).
Round or oval granules, having dark bodies; isolated or united in groups of three or four or in chaplets; very small, difficultly perceptible granules, always united by irregularly marked surfaces.	Soluble in acetic acid, without disengaging gas; insoluble in potassium hydrate.	
Rounded, isolated grains, concentrically striated or radiated (sometimes both); more or less dark and opaque. Small yellowish granules, sometimes very small and in series; sometimes larger, having dark bodies and yellow centres; united in masses, or isolated and covered with spike-like points.	Soluble in acetic acid, with evolution of gas.	Calcium phosphate.
Very small, isolated granules, having a vortex-like motion (Brownian movement).	Slowly soluble in acetic acid, colorless, rectangular tables at the same time appearing.	
Rounded, highly refracting granules of various size.	Insoluble in acetic acid and in potassium hydrate.	Urates (Fig. 38).
	Insoluble in acetic acid; soluble in a mixture of alcohol and ether, especially after the addition of a drop of sodium hydrate.	
		Molecular granules (Fig. 39). Fat globules (Fig. 40).

Fig. 31.



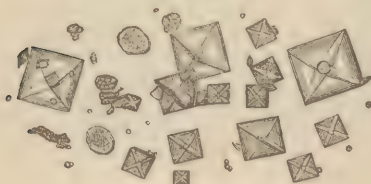
Stellate crystals of triple phosphate.

Fig. 32.



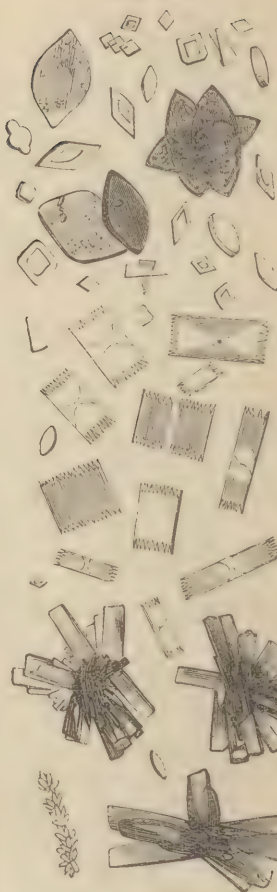
Ammonio magnesium phosphate and crystals of sodium urate grouped in stars.

Fig. 34.



Calcium oxalate.

Fig. 33.



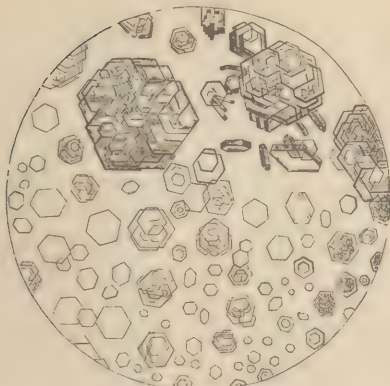
Uric acid.

Fig. 35.



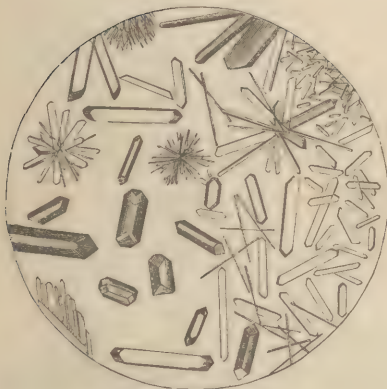
Calcium oxalate.

Fig. 36.



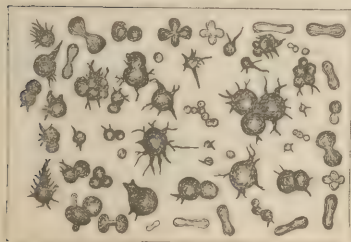
Cystine.

Fig. 37.



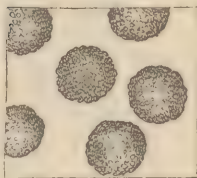
Hippuric acid.

Fig. 38.



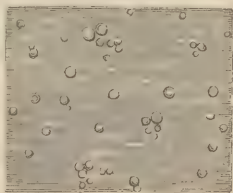
Ammonium urate.

Fig. 39.



Molecular granules.

Fig. 40.

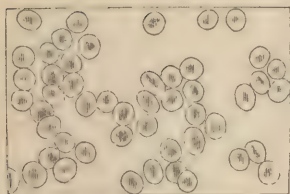


Fat cells.

ORGANIZED SEDIMENTS.

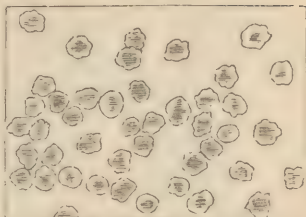
Microscopic characters.	Characteristic reactions.	Substance.
<p><i>a.—Cellular form, more or less rounded.</i></p> <p>Globules, always round, having regular or serrated borders, without nucleus, generally having a central depression, isolated, united in piles, or imprisoned in filaments of fibrin or mucus.</p> <p>Round or oval globules, contours not well marked, contents white, grayish, granular, or nuclear; isolated, or united in masses, in the latter case polygonal, often imprisoned in mucus and elongated.</p> <p>Very small, highly refracting, round, or oval globules; sometimes having one or two brilliant nuclei, or wart-like protuberances; isolated or united in chaplets. (Require a magnifying power of 500 diameters.)</p> <p>Small, refracting, oval, hyaline corpuscles, having a long, delicate, filament-like tail.</p>	<p>Swelled up by acetic acid, or shrivelled like raspberries; not colored by carmine; disappear in potassium hydrate.</p> <p>Rendered paler by acetic acid, which causes the appearance of two or three nuclei; stained by carmine; soluble in potassium hydrate.</p> <p>Not modified by acetic acid, or colored by carmine; the interior nuclei are colored yellow by iodine water.</p> <p>Unchanged by reagents.</p>	<p>Red blood globules (Figs. 41 and 42).</p> <p>Leucocytes (Fig. 43).</p> <p>Spores or organized ferments.</p> <p>Spermatozooids (Fig. 44).</p>
<p><i>b.—Cylindrical or fusiform filaments.</i></p> <p>Rounded, cylindrical, fusiform, or polygonal bodies, having granular contents and more often presenting several nuclei.</p> <p>Large cylinders, having variable appearances; may be long or short; sometimes twisted, or in a wave-like form. (Require 120 diameters.)</p> <p>Cylinders like very small short rods, generally numerous, and all similar; transparent, often agitated with an undulatory movement. (Require 400 or 500 diameters.)</p>	<p>Rendered paler by acetic acid, which makes the nuclei more prominent; colored, especially the nuclei, by carmine.</p> <p>Rendered pale by acetic acid; again developed by alkalis.</p> <p>Not modified by acetic acid, which, however, retards or arrests their movements.</p>	<p>Epithelial cells (Fig. 45).</p> <p>Tube casts from the kidneys (Fig. 46).</p> <p>Vibrios.</p>
<p><i>c.—Filaments or flakes of various appearances.</i></p>	<p>Not changed by acetic acid.</p> <p>Paled by acetic acid, the fibres disappear and give place to a swollen, amorphous, transparent mass, which again becomes fibrous when treated with potassium hydrate.</p> <p>Rendered more prominent by acetic acid, which at the same time produces a dotted or striated appearance.</p>	<p>Algae and fungi.</p> <p>Clots of fibrin.</p> <p>Mucus.</p>
<p>Very thin filaments, differing more or less from each other, and interlaced.</p>		

Fig. 41.



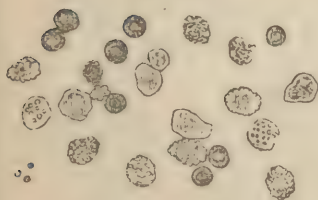
Natural blood corpuscles.

Fig. 42.



Collapsed blood corpuscles.

Fig. 43.



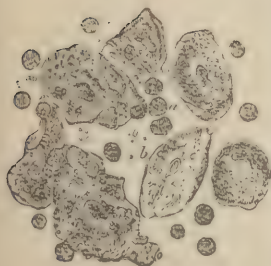
White blood corpuscles.

Fig. 44.



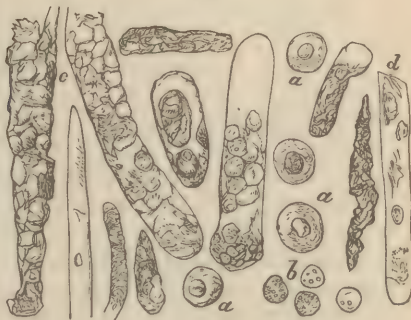
Spermatozooids.

Fig. 45.



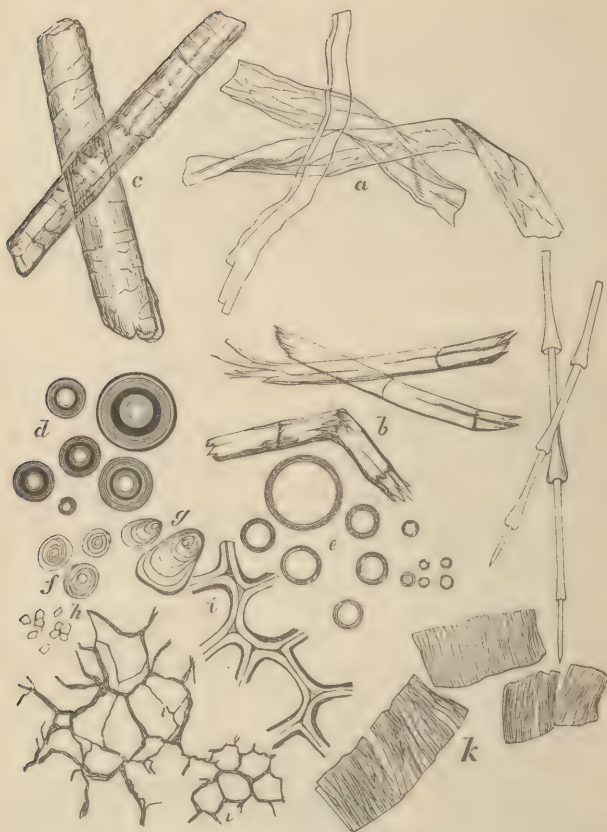
Epithelial and mucus.

Fig. 46.



Casts of the uriniferous tubules.

Fig. 47.

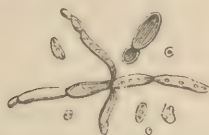


a. Cotton fibres; *b.* flax fibres; *c.* hairs; *d.* air-bubbles; *e.* oil-globules; *f.*, *g.*, *h.*, starch granules; *i.* vegetable structures; *k.* muscular fibres.

As more or less extraneous matters, derived either from atmospheric dust or the vessels in which the urine has been collected, may be expected to be present with the sediment, care must be taken not to confound such matters with the deposit proper. The principal foreign bodies which are likely to be met with are represented in figure 47, taken from *Roberts' Urinary Diseases*.

The more common deposits are ammonio-magnesium phosphate, calcium phosphates, calcium oxalate, and urates; the latter constitute the red or brown sediments, known as brick-dust deposits, so frequently seen in febrile

Fig. 48.



Saccharomyces mycoderma (yeast plant).

urine; their color is due to the pigment of the urine, and depends altogether on the proportion of coloring matter present,—in pale urines they are almost or quite colorless. Ammonium urate is much the more characteristic under the microscope, the spike-like projections being generally well marked. Sodium urate, which seems to constitute the bulk of the red deposits, is in smaller granules having no projections. The two may be distinguished by allowing a drop of hydrochloric acid to penetrate under the thin cover of the microscope slide. Uric acid separates, and at the same time sodium chloride or ammonium chloride crystallizes out; the former crystallizes in cubes, the latter in leaf-like or branched forms.

Calcium oxalate is sufficiently characterized by its crystalline form, and, like the urates, is insoluble in ammonia. The latter property serves for the distinction of urates and calcium oxalate and phosphate from tyrosine, cystine, and xanthine, which are soluble in ammonia.

Uric acid may easily be recognized by the murexide test; tyrosine and xanthine by their behavior when treated with nitric acid and potassium hydrate; cystine by the black stain which it produces when placed upon a silver surface and moistened with potassium hydrate.

Sometimes urine is turbid and even milky from the presence of finely divided fat globules; in this case it is rendered clear and transparent by agitation with ether,

and, when the latter liquid is decanted and evaporated, it leaves the fat in a solid or semi-solid state.

If the urine which is voided by women during pregnancy be allowed to stand for a day or two, a whitish pellicle gradually forms on its surface. This pellicle has a fatty appearance, and was formerly called *k Niestine*. It contains, however, no peculiar principle, but is shown, by a microscopic examination, to consist of ammonio-magnesium phosphate, some fat globules, a few vibrios, and the results of the destruction of epithelial mucus.

Sometimes the microscopic examination of urine reveals the presence of the yeast plant (*saccharomyces mycoderma*). In such a case the urine should always be tested for glucose. The *saccharomyces mycoderma* is represented in fig. 48. It may appear in detached cells, or as a number of buds, clustered together in a sort of chaplet.

The consideration of the organized sediments of urine belongs properly to pathological microscopy.

Urinary Calculi.

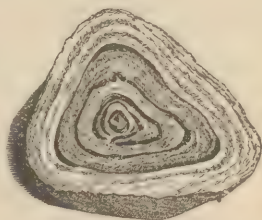
§ 160. The calculi which are sometimes found in the bladder, and indeed throughout the whole urinary tract, differ greatly in their physical appearances and their chemical composition, but always consist of normal or abnormal constituents of the urine. They are as varied as the unorganized urinary sediments which have already been described, and they may be considered as agglomerated sediments. They may be small, like grains of sand; they then constitute *gravel*, and are passed with the urine, producing little or no pain. They may, however, attain great magnitude, and necessitate a surgical operation for their removal. They generally contain a nucleus, around which the calculus is built up in layers, sometimes of the same, sometimes of a different chemical nature. The nucleus may be a crystal of uric acid which has separated spontaneously in the kidney or bladder; it may be a clot of blood, or a tube-cast from the kidney; sometimes, but rarely, it is formed by a foreign body which has been accidentally introduced into the bladder.

A chemical examination of gravel or small calculi which have been passed with the urine, is indispensable, as it may modify the treatment to be adopted, and, in case large calculi exist in the bladder, it may influence the nature of the operation to be performed, and enable the surgeon to decide between lithotritry and lithotomy.

The nature of sand-like calculi may generally be determined by the table of urinary sediments, page 127, and the same chemical tests are applicable to this gravel as those which serve for the identification of larger calculi.

The stone should first be cut through the middle by means of a fine saw; it then immediately becomes apparent whether the calculus is uniform throughout, or whether, as is usually the case, it is made up of different substances in concentric layers (Fig. 49). If the stone be desired for a collection, its nature may be determined, and sufficient material obtained for analysis, by boring a hole in one side.

Fig. 49.



Mixed calculus.

The following mode of operation permits, at the same time, the detection of the constituents of the calculus, and a partial quantitative analysis. If the latter is dispensed with, the weighings are omitted:—

a) Estimation of water.—The stone should be washed with cold water, before crushing, in order to remove the adhering urinary matter; a quantity of the finely pulverized matter is then weighed, dried at 100° in an air-oven, and again weighed. The loss of weight indicates the quantity of water present. A small quantity of ammonia will be volatilized in this operation, if that compound form any considerable proportion of the calculus. Calculi consisting principally of phosphates may contain a large proportion of water, losing even half their weight by desiccation; of course, if the calculus has been allowed to dry for any time in the air before the analysis is made, it will hardly be useful to estimate the water.

b) A small weighed portion of the powder is introduced into a porcelain or platinum crucible, moistened with a drop or two of water, and a few drops of acetic acid are added. Any effervescence denotes the presence of a carbonate.

c) A little strong nitric acid is then added, and the mixture is carefully evaporated to dryness, spreading it out as much as possible on the sides of the crucible. If uric acid be present, the mass acquires a reddish color, which changes to purple on the addition of a drop of very dilute ammonia.

d) The residue is now heated to bright redness, until the whole of the carbonaceous matter is entirely destroyed; if no residue remain, the calculus contains no mineral matter; if a residue be left, its weight is that of the inorganic constituents.

e) If no mineral matter be present, the stone consists of one or more of the following substances:—

Uric acid
Ammonia urate
Xanthine

Cystine.

Organized bodies, such as epithelial matter, pigment, or fibrinous substances.

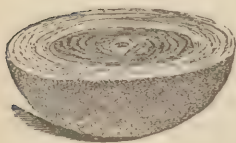
f) The presence of uric acid or ammonium urate is detected by the murexide test (§ 50); the discrimination between the two is made by heating a portion of the powder with a solution of potassium or sodium hydrate. If ammonium urate be present, ammonia will then be disengaged. If, however, in addition to uric acid, the stone contain ammonio-magnesium phosphate, ammonia will be disengaged by the action of an alkaline hydrate,

even though no ammonium urate be present; in this case it cannot easily be decided whether the uric acid be free, or whether it exist in combination with ammonia.

Uric acid is the most common constituent of vesical calculi.

g) Should the murexide test have failed to indicate the presence of uric acid, but the residue of the evaporation of

Fig. 50.



Uric acid calculus.

the nitric acid be yellow, xanthine may be present. In this case the residue will assume an orange color when treated with potassium hydrate, and will then become reddish-violet when heated. (Compare § 52.) Xanthic calculi are of rare occurrence.

h) A stone which is entirely combustible, and contains neither uric acid nor xanthine, may consist of cystine. It will then dissolve in ammonia, and by slow evaporation of the ammoniacal solution the cystine will be deposited in characteristic hexagonal tables, the nature of which can be ascertained by the tests given in section 74.

i) Calculi consisting of albuminoid and epithelial matters are very rare; they disengage an odor of burnt horn when heated, and are soluble in potassium hydrate, the solution responding to the usual tests for albuminoid substances.

k) A stone which is partly combustible and which responds to the murexide test, may contain the urates of potassium, sodium, magnesium and calcium, and possibly calcium oxalate, magnesium or calcium phosphate, ammonio-magnesium phosphate, magnesium or calcium carbonate.

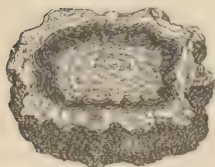
If the presence of an alkaline urate be suspected, the residue of the incineration is exhausted with a little water, and the solution, which would contain the potassium or sodium as carbonate, will have an alkaline reaction; it is tested for potassium by platinic chloride, and for sodium by the flame test.

If the residue of the combustion contain neither potassium nor sodium, it is treated with a little dilute acetic acid, and the solution is neutralized by ammonia, and tested for calcium by ammonium oxalate, and for magnesium by ammoniacal sodium phosphate containing some ammonium chloride. If the presence of either calcium or magnesium be found by these tests, and the original matter effervesce when treated with acetic acid, it cannot be decided whether these metals originally existed as urates or as carbonates; but if no effervescence take place when the original calculus is treated with acetic acid, it may be concluded that an earthy urate is present.

l) To detect calcium oxalate, a portion of the calculus is treated with dilute hydrochloric acid, the solution is filtered, and neutralized with ammonia. The calcium oxalate, which is soluble in hydrochloric acid, is then thrown down unchanged. It is insoluble in acetic acid. When heated, it is converted into calcium carbonate, which dissolves with effervescence in acetic acid; if the heat be raised to redness, the calcium carbonate is re-

duced to lime, and the latter will restore the blue color to moistened red litmus paper.

Fig. 51.



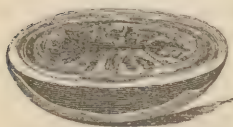
Calcium oxalate calculus.

Stones consisting of calcium oxalate are hard, and covered with rough projections, from which they have received the name of *mulberry calculi*.

m) If the residue of the combustion of the calculus do not effervesce when treated with acetic acid, it consists of one of the phosphates.

Calculi consisting of calcium phosphate are usually smooth and have a polished appearance; they are composed of layers which readily separate from each other when the stone is broken. They are almost infusible.

Fig. 52.



Calcium phosphate calculus.

Calcium phosphate dissolves without effervescence in dilute nitric acid, and is reprecipitated when the solution is neutralized by ammonia. If the precipitate be dissolved in acetic acid, the presence of calcium may be detected by the addition of ammonium oxalate to the solution.

Stones containing ammonio-magnesium phosphate leave on ignition a residue which is fusible, and solidifies to a white, enamel-like mass on cooling. Such calculi disengage ammonia when heated with potassium hydrate.

Ammonio-magnesium phosphate dissolves in dilute hydrochloric acid, and is again precipitated by the

addition of ammonia. The precipitate so formed is crystalline, and may be recognized under the microscope.

Calculi frequently consist of ammonio-magnesium phosphate mixed with calcium phosphate; they are soft, and may be readily crushed. When heated with potassium hydrate, they disengage ammonia. They dissolve in dilute hydrochloric acid, and on the addition of ammonium oxalate, calcium oxalate is precipitated, while magnesium may be detected in the filtered liquid by neutralizing the latter with ammonia; ammonio-magnesium phosphate is then thrown down as a crystalline precipitate.

Phosphoric acid may be detected in all phosphatic calculi, by dissolving them in nitric acid, and adding ammonium molybdate to the solution. Phospho-molybdate of ammonium separates as a yellow precipitate, whose formation is favored by heating the liquid.

n) A calculus which contains calcium or magnesium carbonate, effervesces when treated with acetic acid, and the earthy metal may be detected in the solution obtained. Such calculi are rare in man.

o) Sometimes a calculus contains several of the mineral compounds which have been mentioned, and uric acid or urates in addition. By boiling such a stone (pulverized) with a large quantity of water, most of the uric acid and urates may be removed; from the residue, acetic acid will dissolve the carbonates and phosphates, and the latter are precipitated by the addition of ammonia to the solution. Acetic acid leaves calcium oxalate undissolved; this is, however, soluble in dilute hydrochloric acid, and may be precipitated in its characteristic crystalline form by very slowly neutralizing the solution with ammonia.

B L O O D .

§ 161. The general physical properties of blood are well known; it is a somewhat thick, viscous liquid, having a red color, which is bright scarlet in the arteries and much darker in the veins. It has a faint odor, and a salty, unpleasant taste. The specific gravity of human blood is usually comprised between 1050 and 1058; it may, however, vary from 1045 to 1075 (Gorup-Besanez). It is always alkaline.

Anatomically, the blood is composed of a serous liquid, called the *plasma*, in which are suspended—

Red corpuscles, the characteristic blood-cells;

White globules, or leucocytes;

Small granular masses.

The history of the blood includes a consideration of its anatomical constituents, and of the chemical principles contained in each of the constituents, as well as of the chemical nature of the blood as a mass.

§ 162. *Normal chemical constituents.*—Normal blood contains water, fibrin (fibrinogen and fibrino-plasmin), albumen, hemoglobin, lecithine, cholesterin, and small quantities of urea, glucose, creatine and creatinine, fatty matters and alkaline salts of the fatty acids, and perhaps traces of uric acid. The mineral matters present are alkaline chlorides, carbonates, sulphates and neutral phosphates, calcium and magnesium phosphates, iron and traces of silica.

Besides these bodies, blood holds in solution oxygen, nitrogen, and carbon dioxide.

The hemoglobin, lecithine and iron, exist only in the red corpuscles; the other constituents are partly common to these corpuscles and to the plasma, and in part peculiar to the latter; in the first case are water and the inorganic salts; in the second are fibrin, albumen, fatty matters and salts of fatty acids, cholesterin, urea, glucose, creatine, creatinine, and uric acid.

§ 163. *Abnormal constituents.*—The principal abnormal substances proper which have occasionally been de-

ected in blood, are volatile fatty acids (from formic to butyric), biliary acids and pigments, hypoxanthine, gelatin, lactic acid, leucine and tyrosine, and ammonium carbonate.

All of the substances which may be accidentally or medicinally introduced into the system, and which pass into the blood without immediate change, may also be detected in the blood; among these substances are the metallic poisons, alkaloids, hydrocyanic acid, carbon monoxide, etc.

General Chemical Properties.

§ 164. Shortly after blood is drawn from the vessels, it coagulates, forming a solid mass; it also coagulates in the vessels, if from any cause its circulation be arrested. The coagulation of drawn blood is hastened by agitation, and is retarded or even completely arrested by the presence of an alkaline hydrate or carbonate, or of traces of mineral or organic acids, or by certain salts, among which may be particularly mentioned sodium sulphate, potassium nitrate, and common salt.

If coagulated blood be allowed to stand, the clot gradually contracts in volume during a day or more, and at the same time a viscid liquid called the serum is pressed out of the contracting mass.

When freshly drawn blood is allowed to stand, without any agitation, the suspended globules have a tendency to sink to the bottom of the containing vessel, their specific gravity being greater than that of the serum. The upper layer of the mass then becomes almost colorless by the time that the blood solidifies, and this slightly concave, almost transparent, firm and elastic upper layer imprisons the greater part of the white corpuscles. It has been called the *buffy-coat*. The lower portions of the clot are softer, less elastic, and contain the greater mass of the red corpuscles. The larger the proportion of fibrin in the blood, the more voluminous is the buffy-coat, but the same effect is observed when the blood is deficient in red corpuscles; in the latter case, however, the clot is usually quite small, and floats on the serum when the latter separates. When the blood is very rich

in red corpuscles, the buffy-coat is not well marked, and the clot is abundant.

If, instead of being allowed to stand, freshly drawn blood be agitated by the aid of a glass rod, or a bundle of twigs, the fibrin coagulates on the rod or the twigs, in colorless, elastic filaments.

By mixing blood, thus freed from its fibrin, with five or six times its volume of a cold saturated solution of sodium sulphate, a liquid is obtained from which the red corpuscles may be separated by simple filtration, the liquid which passes through the filter being nearly colorless. This liquid coagulates in a mass when heated, by reason of the albumen which it contains. Freshly drawn blood also coagulates into a sort of paste when mixed with alcohol, mineral acids, or other agents which coagulate albumen.

Carbon monoxide expels the oxygen from the hemoglobin of blood, coloring the latter violet-red; oxygen will not readily expel the carbon monoxide which is thus absorbed. This fact explains the poisonous action of carbon monoxide.

If dried blood be mixed with a little sodium chloride, and then heated to boiling with glacial acetic acid, a brownish-red liquid is obtained, which soon becomes almost black, and deposits crystals of hemin as a dark, brilliant powder.

The serum of blood, as it separates from the clot, has a color varying from yellow to reddish; it is generally alkaline, and coagulates on being heated. It is precipitated by alcohol, mineral acids, and many metallic salts. When serum is neutralized by the addition of a few drops of acetic acid, and then poured into boiling water, the albumen coagulates in large flakes which may be easily separated by filtration.

The residue obtained after thoroughly incinerating dried blood, consists of potassium and sodium chlorides, potassium, sodium, calcium, and magnesium phosphates, iron, traces of other metals, and a little silica; there are also present alkaline carbonates, produced by the combustion of alkaline salts of organic acids. These salts are contained almost wholly in the clot; the serum

contains only a very small proportion, its ash consisting principally of the alkaline base which exists in combination with the albumen.

Analysis of Blood.

§ 165. The chemical examination of blood may be undertaken either for the detection of certain normal or abnormal constituents, or for purposes of research, or for the estimation of the proportions of one or more of the elements present.

Those substances which are contained in the blood in tolerably large proportions, may be detected by methods which have already been indicated when treating of the general properties of such compounds. For some bodies, however, which are usually present in very minute quantities, special precautions must be observed. These bodies are best detected in the serum, and to obtain the latter, as large a quantity of the blood as possible should be allowed to coagulate, and the clear liquid is separated from the clot by decantation.

§ 166. UREA.—For the detection of urea, the clear serum is mixed with three or four times its volume of strong alcohol, and allowed to stand several hours, after which it is filtered, and the filtrate evaporated nearly to dryness on a water-bath. The residue is then exhausted with absolute alcohol, and the extract so obtained is filtered and again evaporated nearly to dryness. The residue is dissolved in a small quantity of water, and the solution filtered; the filtrate is freed from phosphates by the addition of baryta-water, and the excess of barium hydrate is precipitated by a current of carbon dioxide. The clear liquid obtained by filtration is concentrated to a syrupy consistence, and the vessel containing it is then placed in ice-water, or in a very cold place, and a few drops of concentrated nitric acid are added. After standing some time, the solution deposits crystals of urea nitrate, which may be identified by the properties described in section 41.

§ 167. URIC ACID.—As large a quantity as possible of the serum is freed from albumen by boiling it with two

or three times its volume of water to which a few drops of acetic acid have been added. The mixture is filtered through a cloth, and the filtrate is evaporated to dryness on a water-bath. The residue is exhausted several times with boiling water, the united extracts are filtered while boiling, and the filtrate is evaporated to a small bulk, mixed with a little strong acetic acid, and allowed to stand several days. The crystals which separate are then examined microscopically, and submitted to the chemical tests indicated in section 50.

Uric acid may also be separated from serum of blood by the method described in section 51.

§ 168. CREATINE AND CREATININE.—The albuminous matters are removed from the serum by boiling with water and a few drops of nitric acid; the filtrate is precipitated by basic lead acetate, the liquid again filtered, and the filtrate freed from excess of lead by hydrogen sulphide. The clear filtered solution is evaporated to a small bulk on a water-bath, the residue is exhausted with absolute alcohol, and the alcoholic solution mixed with a neutral solution of zinc chloride, and the operation continued as in section 59.

§ 169. GLUCOSE.—The serum, or defibrinated blood, is mixed with four times its volume of alcohol, and after standing a few hours the mixture is filtered. A few drops of acetic acid are added to the filtrate, which is then heated to boiling, and again filtered; the new filtrate is evaporated to dryness on a water-bath. The residue is exhausted with a little warm water, and the solution is tested for glucose according to the methods indicated in sections 21–24, preferably by means of Fehling's solution.

§ 170. MINERAL SALTS must be sought in the ash obtained by evaporating to dryness and carbonizing about 50 c.c. or more of the blood. The carbonaceous mass is exhausted with warm water, the liquid filtered, and evaporated to dryness; the residue consists of the soluble mineral salts, and these are detected by the usual chemical tests. Silver nitrate added to the solution produces a white precipitate, indicating the presence of chlorides; barium chloride precipitates the sulphates:

ammonia, ammonium chloride, and magnesium sulphate, together, precipitate the phosphoric acid. The carbonaceous residue from which the soluble salts have been extracted, still contains the insoluble salts. It may be incinerated, and the salts remain as a brownish ash, which will dissolve in boiling hydrochloric acid; iron may be easily detected in this solution.

§ 171. BILIARY ACIDS AND PIGMENTS, if present, may be detected by Pettenkofer's and Gmelin's tests, which have already been described (§§ 135 and 136).

§ 172. LEUCINE AND TYROSINE.—These bodies appear to be present in blood only in acute diseases of the liver; for their detection, the serum or defibrinated blood is poured into boiling water, which precipitates the albumen, and the filtered liquid and wash-water are evaporated to about one-third the volume of the blood taken. Basic lead acetate is then added, and the subsequent steps of the operation are conducted according to the indications given in section 140.

§ 173. AMMONIA.—The following method, proposed by Brücke, depends upon the use of Nessler's reagent; the latter is made by dissolving 2 grammes of potassium iodide in 5 c.c. of distilled water, warming the liquid, and adding mercuric iodide as long as it continues to be dissolved. After cooling, the solution is diluted with 20 c.c. of water, allowed to stand several hours, filtered, and 20 c.c. of the filtrate are mixed with 30 c.c. of a concentrated solution of potassium hydrate, as free as possible from potassium carbonate. If the liquid become turbid, it must again be filtered, and no ammonia should be present in the atmosphere of the room in which it is prepared.

Some of the blood to be examined is then introduced into a small flat dish which may be hermetically closed by a glass plate. A very small porcelain capsule is fastened to the inside of the glass cover by the aid of a little wax, and a few drops of very dilute sulphuric acid are spread out over its surface. The cover is then oiled on the edges, and placed over the vessel containing the blood, and the whole is allowed to stand for an hour or longer in a tolerably warm place. The cover is then

removed, and a drop or two of Nessler's reagent is poured into the capsule which had been moistened with sulphuric acid. If ammonia has been disengaged, it will have been absorbed by the sulphuric acid, and a reddish-brown color is produced on the addition of Nessler's reagent.

§ 174. CARBON MONOXIDE may be detected in the blood after poisoning by that gas. The spectroscopic characters of oxyhemoglobin and of hemoglobin containing carbon monoxide, have already been indicated (§ 87). The blood to be examined is properly diluted with water, in order that it may not be too opaque, and is placed in a small glass trough having parallel plane glass sides, about one centimetre apart. The rays of light from a gas jet or oil lamp are caused to traverse this solution perpendicularly before entering the narrow slit of the spectroscope. The absorption bands then produced by blood containing carbon monoxide much resemble those produced by oxyhemoglobin, but the band immediately to the right of the D line is somewhat nearer E (see fig. 23). When, in such a case, the blood is treated with reducing agents, such as ammonium sulphide, the absorption bands do not disappear, nor does the absorption band of reduced hemoglobin become visible. If no carbon monoxide be present, and the blood be treated and examined as directed, the absorption bands of oxyhemoglobin rapidly disappear, being replaced by the wide and less marked band characteristic of reduced hemoglobin.

Quantitative Analysis.

§ 175. The blood being a mixed liquid, the quantitative estimation of its constituents may be directed specially to the globules, which together with the fibrin form the clot, or to the plasma, which represents normal blood without its corpuscles, or to the serum, which contains neither globules nor fibrin, and very little mineral salts.

The following table, by Becquerel and Rodier, is intended to represent the average composition of human blood:—

Density	1060
Water	781.60
Globules	135.00
Albumen	70.00
Fibrin	2.50
Fatty, extractive, and saline matters	10.00
Phosphates	0.35
Iron	0.55
	<hr/> 1000.00

It must be remembered, however, that the exact estimation of all of the constituents of blood, that is, of the proximate principles, globules, salts, fatty matters, etc., is almost impossible, and complete analyses can, therefore, only be regarded as giving approximate results.

We will first consider a general plan by which a tolerably accurate idea of the constitution of the blood may be obtained, and will then take up specially some of the constituents more difficult to estimate.

The blood is divided into three portions immediately after it is drawn.

20 grammes are reserved for the estimation of water, solid matters, and mineral salts.

At least 40 grammes are beaten up with any suitable appliance for the separation and estimation of fibrin.

40 grammes are allowed to coagulate in a rather flat, covered vessel; the serum is then decanted into a platinum capsule, and dried at 100°. The weight of the residue is that of the albumen plus certain mineral salts, and extractive and fatty matters. The latter are estimated separately, and their weight being deducted from that of the impure albumen, the proportion of pure albumen is obtained. The total proportion of water in the blood having already been determined, as indicated farther on, the amount of albumen corresponding to the water so estimated is calculated, supposing all of the water to enter into the composition of the serum, and the fibrin, globules, and salts to be perfectly dry.

In the same manner the weight of the dry globules is estimated indirectly; the total weight of the organic matter in 1000 parts of serum being known, together with that of the fibrin and salts in 1000 parts of blood, and

the total weight of the solid matter in 1000 parts of blood being known, the difference will represent the dry globules.

While, as has been said, this method can only be approximate, it may be useful, in certain cases, to indicate which part of the blood is deficient or in excess. An example will elucidate the method of calculation.

The estimation of water and of fixed matters has shown 1000 grammes of blood to contain—

Water	788.50 grammes.
Solid matter	{	organic	:	202.34	}	211.50
		mineral	:	9.16	}	"

The determination of fibrin has demonstrated the presence of 2.352 grammes of that substance in 1000 of blood.

The serum of the third portion of blood has been found to consist of,

Water	917.28 grammes.
Solid matter	{	organic	:	75.63	}	82.72
		mineral	:	7.09	}	"

The serum corresponding to 788.50 grammes of water, or 1000 grammes of blood, would then contain 82.72×788.5 or 71.11 grammes of impure albumen,

917.28

which would be composed of

Albumen and extractive matters	65.01	}	71.11 grammes.
Mineral salts	6.11	}	

The sum of the weights of the fibrin and salts contained in 1000 grammes of blood, and of the organic matter in the serum of 1000 parts of blood, is

2.352 + 65.01 + 9.16.	= 76.522 grammes.
The solid matter in 1000 grammes						
of blood	= 211.500

Hence the dry globules would weigh 134.978

The analysis has thus shown 1000 grammes of blood to contain

Water	788.500 grammes.
Fibrin	2.352
Albumen and extractive matters	65.010
Corpuscles	134.978
Mineral salts	9.160

§ 176. ESTIMATION OF WATER AND OF SOLID MATTERS.

—In order to obtain accurate results in the estimation of water, the blood must not be allowed to undergo any evaporation between the time at which it is drawn and the moment of weighing. It is, therefore, necessary to receive the blood directly in a small bottle, which can be corked immediately, when a sufficient quantity has been obtained. The bottle and its contents are then weighed and the previously determined weight of the bottle is deducted. The bottle is then well shaken, in order to break up the fibrin, and the blood is poured out into the platinum capsule in which it is to be dried. The bottle is washed out with a little diluted water, which is added to the contents of the capsule. The latter is then dried in an air oven at 100° , until its weight becomes sensibly constant. The dry residue is quite hygroscopic, and the capsule should, therefore, be covered during the weighings. The desiccation is not perfect at 100° , but if the temperature be raised much higher, some of the constituents of the blood may undergo partial decomposition. Hence if a more thorough desiccation be desired, it is safer to effect it by continuing the drying over sulphuric acid in a vacuum.

The residue in the capsule represents the total solid matter in the quantity of blood desiccated: the difference between the weight of the latter quantity, and that of the residue, is the weight of the water.

§ 177. ESTIMATION OF THE MINERAL SALTS.—The total proportion of mineral salts present, cannot be accurately determined in one operation, for a considerable proportion of the chlorides might be volatilized by the high temperature necessary for the destruction of the last traces of carbon. The soluble salts and insoluble salts, are therefore estimated separately, and for this purpose the residue obtained after estimating the water and fixed matters is cautiously heated over a lamp until it is thoroughly carbonized, and the carbon begins to burn. The black mass is then completely exhausted with warm water, and the solution so obtained is evaporated to dryness in a platinum capsule; the white residue is heated to dull redness, and after cooling is weighed, its weight being that of

the soluble salts. The carbonaceous mass which has been exhausted with water is dried, and if it has been collected on a filter, is returned to the capsule, together with the filter; the whole is completely incinerated, and the weight of the filter-ash is deducted from that of the brownish-residue. The weight of the insoluble salts so found is added to that of the soluble salts previously determined.

§ 178. ESTIMATION OF FIBRIN.—The blood should be collected and beaten in a small precipitating glass or beaker, to the top of which is adapted a caoutchouc cover, through which passes the handle of a whale-bone agitator. (Fig. 53.) When the cover is in position, the lower part of the agitator, which is somewhat expanded, should almost touch the bottom of the vessel. The total weight of this apparatus, empty and quite dry, is determined before the analysis. (Hoppe-Seyler.)



30 or 40 grammes of the blood to be analyzed are collected in the vessel, directly from the vein; the cover is immediately replaced, and the blood is agitated for ten or fifteen minutes. The whole is then allowed to cool, and the weight of

the blood taken is determined by deducting the weight of the empty apparatus from the total weight of apparatus and blood. No loss takes place during the cooling, as the caoutchouc cover prevents sensible evaporation.

The cover is then removed, the vessel nearly filled by pouring in distilled water, and the whole is well agitated. The fibrin deposits in flakes; the transparent supernatant liquid is decanted into another vessel, and the fibrin is shaken up with a fresh quantity of water, containing a trace of common salt. The mixture is then poured upon a small filter which has been dried at 110° and weighed. By the aid of a clean pair of forceps, any filaments of fibrin adhering to the whalebone beater are removed and

added to that on the filter. The mass is then thoroughly washed with pure water,—most efficiently by the aid of a filter-pump,—until the wash-water passes through colorless, and nearly all the color of the fibrin has been removed. The washing is then repeated two or three times with boiling alcohol, to extract fatty matters, and the residue is dried at 110° in an air-oven, until its weight becomes constant. It is then weighed between two watch glasses.

The coagulated fibrin may also be washed by collecting it in a cloth of fine black silk, and tying the latter around it in a tight knot, as soon as the liquid has drained off. The mass is then well kneaded in a stream of water, and finally washed in hot alcohol; on opening the silk, the fibrin will be found almost white. By the aid of a small pair of forceps and a lens, the last particles of fibrin may be removed from the silk, and placed directly in the watch glass in which they are to be dried and weighed.

Normal blood contains about 2.5 grammes of fibrin per kilogramme, the arterial blood being a little richer in fibrin than the venous blood.

If it be desired to estimate the proportion of fibrin in a clot, the latter is divided into small portions, one of which is tied in a knot in a black silk cloth, and thoroughly washed under a jet of water. When all except the fibrin is washed out, another portion of the clot is added, the washing continued, and the operation repeated until the whole of the clot has been converted into a white or nearly white mass. In order that no fibrin may be lost, the silk employed must be sufficiently fine.

§ 179. If only a small quantity of blood is attainable, the proportions of water, of solid matters, and of mineral salts, may be estimated in part of the defibrinated blood, which must then be filtered directly from the fibrin, without addition of distilled water. In this case, three or four grammes of the defibrinated blood are rapidly weighed in a small porcelain capsule of which the weight when empty and dry has been accurately determined. The whole is then dried at $100\text{--}110^{\circ}$ in an air oven, until it undergoes no further diminution in weight.

From the weight of the residue, the proportion of solid matter in 1000 parts of the defibrinated blood is calculated; then the proportion in the natural blood; and if the proportion of fibrin in 1000 parts of blood be added to the result so obtained, the sum will represent the total solid matter in 1000 parts of the blood; of course, 1000, less the total fixed matter, will represent the proportion of water in the blood.

The residue in the porcelain capsule may now be calcined for the estimation of the mineral salts in the defibrinated blood, and their proportion in the natural blood is then found by a simple calculation.

The following example explains the method of calculation.

Estimation of fibrin.

Weight of apparatus in which the blood is beaten, together with the blood	71.471 grammes.	
Weight of apparatus alone	33.840	"
	<hr/>	
Weight of blood	37.631	"
	<hr/>	
Weight of watch glasses with the dry fibrin	5.254	"
" " " alone	5.165	"
	<hr/>	
Weight of fibrin	0.089	"

Hence $\frac{.089 \times 1000}{37.631} = 2.36$ grammes, the amount of fibrin contained in 1000 grammes of blood.

Estimation of fixed matters, water, and mineral salts.

Weight of porcelain capsule with defibrinated blood	21.397 grammes.	
Weight of porcelain capsule alone	17.865	"
	<hr/>	
Weight of defibrinated blood	3.532	"
	<hr/>	
Weight of capsule with dry residue	18.628	"
" " alone	17.865	"
	<hr/>	
Weight of residue	0.763	"

Hence $\frac{763 \times (1000 - 2.36)}{3.532} = 213.08$, the proper-

tion of solid matter in 1000 parts of blood, in addition to the fibrin. The total solid matter and water, are calculated by adding the fibrin and other fixed matters together, and deducting the sum from 1000. Thus—

1000 parts of blood contain . . .	2.36 parts of fibrin.
“ “ “ . . .	216.08 “ other solid mat- ters.
<hr/>	
The total amount of solid matter =	218.44
“ “ “ water =	781.56
The capsule with the residue after incineration	
weighs	17.904 grammes.
The capsule alone weighs	17.865 “
<hr/>	
The mineral salts in 3.532 grammes of defibrinated	
blood weigh	0.039 “

Consequently, the mineral salts in 1000 parts of blood will equal

$$\frac{.039 \times (1000 - 2.36)}{3.532} = 11.01$$

Water	781.56
Fibrin	2.36
Mineral salts	11.01
Albumen and other solid matters (by difference)	205.07

Analysis of Serum.

ESTIMATION OF ALBUMEN, SALTS, ETC.

§ 180. 4 or 5 grammes of serum are exactly weighed in a tared vessel, and poured into about 20 cubic centimetres of boiling distilled water; the vessel is washed out several times with a little cold water, which is then added to the mixture of serum and water. By the aid of a glass rod, a few drops of acetic acid are added to the boiling mixture until the albumen coagulates in large flakes; care must be taken to use enough acid, and also to avoid an excess; if too small a quantity be employed, the albumen does not separate well, and in presence of an excess, the liquid remains milky, and part of the albumen may be redissolved.

The mixture is then thrown on a filter, and the precipitate thoroughly washed with water, the filtrate and washings being retained for the subsequent estimation of

soluble salts and extractive matters. The coagulated albumen is removed from the filter while still moist, which may readily be accomplished by the aid of a small spatula, and transferred to a small tared watch glass, dried at 100 or 110°, and weighed.

The filtrate and washings may be evaporated to dryness in a small porcelain capsule on a water bath, and the residue dried at 100 or 110°, and weighed. This residue consists of soluble salts and extractive matters; the former are estimated by cautiously igniting the mixture over a lamp, until the carbon is completely consumed, and weighing the ash. The proportion of soluble salts so found is deducted from the total mineral salts contained in the serum, as determined by another operation, and the insoluble salts are thus estimated.

§ 181. Hoppe-Seyler recommends the coagulation of the albumen by alcohol, the process being as follows: Between 20 and 50 grammes of the serum are mixed with three or four times their volume of alcohol, and the mixture is allowed to stand in the cold for several hours. It is then filtered, and the precipitate washed, first with absolute alcohol, then with alcoholic ether, and finally with hot water, the liquids being collected separately. The albumen and insoluble salts alone are left upon the filter, provided the serum employed was quite clear. The liquids used for washing carry with them a small proportion of the albumen, which is subsequently coagulated and added to the first portion, as will shortly be indicated. The entire coagulated albumen is again washed with alcohol, to remove the water with which it is impregnated, and is then dried at 110°, and weighed. The residue is ignited in a porcelain crucible, and the remaining ash represents the insoluble salts of the serum.

In washing the albumen, three liquids have been obtained; (1) an alcoholic solution; (2) an alcoholic-etheral solution; (3) an aqueous solution.

(1) is evaporated nearly to dryness on a water-bath, and the residue is mixed with solution (2); the whole is then filtered, and the insoluble portion is washed, first with alcohol, then with alcoholic ether, and finally with the aqueous solution (3), the filtrates being collected

separately as before. The residue is repeatedly washed with small quantities of water, and then consists of the albumen which was dissolved in the first operations. It is added to the albumen at first coagulated, or may be dried, weighed, and ignited in the same manner separately, if so desired.

The aqueous solution (3) contains all of the soluble salts, except those which are soluble in alcohol and ether; it is evaporated to dryness, the residue is exposed to a temperature of 110° in an air-oven, and weighed. It is then ignited at a red heat, and again weighed. The final residue consists of soluble mineral salts, while the difference between the two weighings is attributed to extractive matter.

The alcoholic-etheral solution (2) contains urea, glucose, a little sodium chloride, cholesterin, fatty matters, and lecithine. It is evaporated at a temperature below 70° , or better, in a vacuum. The residue is exhausted with ether, thrown on a filter, repeatedly washed with ether, and the washings are added to the ethereal extract. The filter is then broken, and the residue washed into a small capsule in which it is desiccated at 110° , and weighed. It is then incinerated, and again weighed.

The ethereal solution is distilled in a small retort or flask, on a water-bath, and the residue is dried in the air-oven, and weighed as rapidly as possible. It is then treated with alcohol, and an alcoholic solution of potassium hydrate, and digested on the water-bath until all of the alcohol is expelled. The residue, consisting of a mixture of soaps, cholesterin, neurine, calcium phosphoglycerate, glycerin, and potassium hydrate, is treated with water, and agitated with an equal volume of ether; after the latter has separated, it is decanted, and the treatment with ether repeated several times. The ethereal liquids are united, and the ether distilled off in a small retort, the residue being evaporated to dryness. This residue consists of impure cholesterin; it is exhausted with a small quantity of absolute ether, the new ethereal solution is evaporated, and the residue dried and weighed. It is almost pure cholesterin. The aqueous residue of the agitation with ether contains potas-

sium soaps of the fatty acids, the excess of potassium hydrate, etc. It is evaporated to dryness, and the residue mixed with potassium nitrate and completely incinerated in a platinum crucible. The mass is then exhausted with water, and an excess of nitric acid is added. The solution is evaporated to a small bulk, mixed with ammonium molybdate, and allowed to stand for about twelve hours; the yellow precipitate of ammonium phospho-molybdate is dissolved in dilute ammonia, and the solution is treated with magnesium sulphate, ammonia, and ammonium chloride, and set aside for twenty-four hours. The precipitate of ammonio-magnesium phosphate is then collected, dried, and converted into magnesium pyrophosphate by strong ignition. The weight of the pyrophosphate multiplied by 7.2748 gives the weight of the lecithine from which the phosphorus was derived.

This method of Hoppe-Seyler permits the estimation—

1) Of albuminoid matters and insoluble mineral salts. The difference between the weight of the impure albumen and that of its ash, is considered as the weight of the pure albumen.

2) Of soluble mineral salts, these being represented by the united ashes of the aqueous and alcoholic solutions.

3) Of extractive matters, soluble and insoluble in alcohol.

4) Of extractive matter soluble in ether, with special estimations of cholesterin, fatty matters (by difference), and lecithine.

The quantities thus found are calculated for 1000 parts of serum.

According to Becquerel and Rodier, 10 grammes of extractive matters of blood, including both fatty and soluble substances, contained—

Serolin	0.025
Soaps of fatty acids	1.400
Cholesterin	0.125
Sodium chloride	3.500
Soluble salts of sodium	2.500
Indefinite matters	2.450

ESTIMATION OF HEMOGLOBIN.

§ 182. The most practicable, and most exact method for the estimation of hemoglobin, consists in determining the proportion of iron in the ash of a known quantity of blood. The estimation of the iron is most rapidly effected by the volumetric method depending upon the oxidation of a ferrous salt by a standard solution of potassium permanganate.

About one gramme of pure potassium permanganate is dissolved in one litre of distilled water, and the exact strength of the solution is determined by the aid of a solution of ferrous chloride, made by dissolving one gramme of pure iron wire in hydrochloric acid in a narrow-necked flask, and diluting the liquid to one litre. 10 c. c. of this solution, containing 10 milligrammes of iron, are measured into a beaker of about 150 c. c. capacity, and the volume is made up to about 50 c. c. with distilled water.

A burette provided with a glass stopcock is filled to the 0 division with the permanganate solution, which is then added drop by drop to the solution of ferrous chloride, until the latter acquires a persistent rose-tint. The volume of permanganate required corresponds to 10 milligrammes of iron.

50 or 100 grammes of the blood in which the hemoglobin is to be estimated, are evaporated to dryness, and the residue is completely incinerated in a platinum or porcelain capsule. The ash is dissolved in dilute hydrochloric acid, most of the free acid expelled by evaporation, and a few pieces of pure zinc are placed in the solution until the latter becomes entirely colorless; in this manner the ferric chloride formed is reduced to ferrous chloride. The zinc is then removed, and the liquid is diluted to about 50 c. c. with distilled water. The solution of potassium permanganate is then slowly added from the burette until all of the iron is converted into ferric salt, as is indicated by the rose-color of the solution. This rose-color gradually fades after a time, but this effect is not due to the oxidation of the iron. As the exact volume of permanganate required to oxidize 10

milligrammes of iron is known, the amount of iron in the ash is easily calculated from the volume of permanganate required to oxidize its solution. The proportion of iron thus found, multiplied by 238.1, gives the proportion of hemoglobin; the latter compound, according to the analyses of Hoppe-Seyler, contains 0.42 per cent. of iron.

This method is accurate, provided great care be exercised in the manipulation, and in originally determining the strength of the potassium permanganate solution. As the latter undergoes certain changes in time, its exact strength should be found shortly before the analysis.

Example.—It is found that 6.2 c.c. of the permanganate solution are required to produce a persistent rose tint when added to 10 c.c. of the ferrous chloride solution, properly diluted as directed. Consequently, 1 c.c. of the permanganate will oxidize $\frac{10}{6.2}$ milligrammes of iron from the ferrous to the ferric condition.

100 grammes of blood are evaporated and incinerated, the residue is dissolved in dilute hydrochloric acid, the solution reduced by metallic zinc, and then diluted to 50 c.c. This liquid being titrated with the permanganate solution, it is found that 26.8 c.c. of the latter are required to produce a permanent rose-color. But 6.2 c.c. of permanganate have been found equivalent to 10 milligrammes of iron.

Then $6.2 : 10 = 26.8 : x$, or $\frac{26.8 \times 10}{6.2} = 43.2$ milligrammes of metallic iron are found in 100 grammes of blood. Hence, the latter contains 43.2×238.1 , or $43.2 \times 100 = 10.285$ grammes of hemoglobin.

0.42

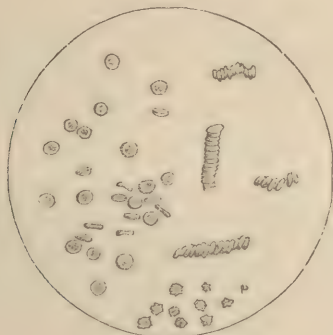
Anatomical Analysis of Blood.

§ 183. If freshly drawn blood be examined under the microscope before it coagulates, it will be seen to consist of a transparent, colorless fluid, in which float a multitude of small disks, having a pale yellow color. These

are the red corpuscles to which the color and opacity of the blood is due ; their pale color under the microscope is caused by the fact that the rays of light pass through single corpuscles, and are spread over a large surface.

The corpuscles of human blood have an average diameter of six- or seven-thousandths of a millimetre, and a thickness of about two-thousandths of a millimetre. They are nearly circular, and exhibit a slightly depressed or concave centre. They have a great tendency to agglomerate together in *rouleaux*, like piles of coin (Fig. 54).

Fig. 54.

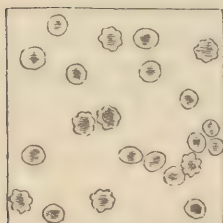


Red blood-corpuscles.

When placed in liquids of different densities, the corpuscles undergo various changes in form and dimensions. In a solution having about the same specific gravity as their natural plasma, they remain unchanged ; a one per cent. solution of common salt fulfils the required conditions, and such a liquid may be employed to preserve the form of the corpuscles in microscopic examinations. If the liquid be less dense than the plasma, for instance, if the corpuscles be placed in pure water, they gradually become distended and globular, and finally burst ; if, on the other hand, the liquid have a greater density than the plasma, the corpuscles become wrinkled, and collapse. This latter change of form frequently takes place while fresh blood is being examined between two glass slides by the aid of the microscope, and is then due

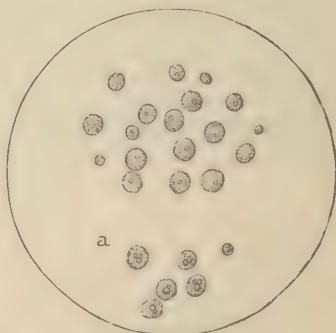
to the gradual evaporation by which the surrounding liquid becomes concentrated (Fig. 55).

Fig. 55.



Blood corpuscles undergoing collapse.

Fig. 56.



White blood-corpuscles.

In addition to the red corpuscles, the blood contains a much smaller number of white corpuscles, leucocytes, having somewhat irregular forms, and a granular appearance (Fig. 56). They are identical with the white corpuscles of lymph, chyle, and pus. When treated with acetic acid, they become transparent, and are seen to contain one or more nuclei.

The blood-corpuscles are dissolved and destroyed by strong alkaline and acid liquids, but, if blood has simply dried, the corpuscles retain their identity, and often, even after the lapse of years, they may be recognized by their form and properties (see farther on).

The density of the red corpuscles is 1088, considerably greater than that of the plasma in which they float; according to Denis, the weight of the dry globules is to that of the water which they contain as 1 is to 1.8.

Although the corpuscles are solid or semi-solid substances, and only suspended in the plasma, they cannot be separated from the latter by filtration, for they readily pass through the pores of filter-paper, being exceedingly elastic, and able to change and reassume their form. The addition of a large proportion of a saturated solution of sodium sulphate or magnesium sulphate to the blood,

however, renders the corpuscles less elastic; their borders become serrated, and the central depression more distinctly marked. The mixture may then be filtered through paper which has previously been moistened with a saturated solution of sodium sulphate, and the corpuscles will remain on the filter. The mass may be washed with a saturated solution of sodium sulphate, drained as thoroughly as possible, and weighed. Of course, results obtained in this manner are only roughly approximate.

Detection of Blood Stains and Spots.

§ 184. It is frequently necessary, in medico-legal investigations, to determine whether spots upon clothing, or other fabric, or upon articles of furniture, wood, or instruments with which it is supposed a crime has been committed, are or are not due to blood. Sometimes it is also required to distinguish between human blood and that of another animal, but in this case the question can only be considered by a skilled microscopist, and one who has long and carefully studied the forms and diameters of the blood-corpuscles of different animals, and the crystals which may be obtained from different kinds of blood.

In most cases, however, it is not difficult to decide simply whether the spots be due to blood, unless they have been mixed with materials which profoundly modify the nature of the constituents of blood.

The detection of blood is effected by microscopical examination, and by chemical tests.

The microscopic examination depends upon the identification of the red corpuscles. It is, therefore, necessary to bear in mind the modifications which these corpuscles undergo under the action of different reagents. Water causes them to become spherical and transparent, by swelling them and dissolving their coloring matter. Hence the spots should never be washed with water before the microscopic examination. As has already been mentioned, they are altered and destroyed by acids, alkalies, chloroform, ether, etc.

In order to restore the characteristic forms of the corpuscles in dried blood, the spot is moistened with a liquid whose specific gravity is about that of the plasma, and which will preserve the corpuscles. An artificial serum may be made by dissolving 30 grammes of white of egg and 40 centigrammes of sodium chloride in 270 grammes of distilled water; or a solution containing 5 or 6 decigrammes of either sodium chloride or sodium sulphate in 100 grammes of water, may be employed. The part of the fabric, paper, wood, or other material containing the spot, is cut out, and soaked in a few drops of one of the above liquids in a watch-glass, or directly on a microscope slide. The glass is then covered, and allowed to stand until the spot is entirely softened; but little time is required if the stain be recent, but, if it be very old, a day or two may be necessary.

When the spot seems completely disintegrated, the liquid is examined by the aid of a microscope, a high power being employed. When the blood has not long been dried, sufficient red corpuscles may always be observed to render their identification easy and certain, but in old spots only a few corpuscles are generally found, the greater number having been destroyed by the desiccation. The corpuscles recovered from dried blood sometimes present their normal color, form and diameter, sometimes they are almost colorless, nearly spherical, or shrunken and serrated on the edges, and smaller than when in the normal condition. Hence it is often impossible to identify the animal from which the blood was derived.

However, while the microscopic examination may be indecisive, owing to the desiccation and destruction of the blood-corpuscles, the application of certain chemical tests will generally remove all doubt upon the nature of suspected blood-stains.

The tests depending upon the detection of albuminous matter and of iron and ammonia in the spots, may be used, if desired, as corroborative evidence, as may the reaction with tincture of guaiac, but these tests are not conclusive, and could not be relied upon alone. It is

not so with the tests by the aid of which, hemoglobin and hematin are recognized; nor with the hemin test, by which crystals of the latter body are obtained.

§ 185. *Detection of hemoglobin and of hematin.*—The absorption bands of oxyhemoglobin are usually apparent in the absorption spectrum of blood, when the latter is comparatively recent. The dried blood, or the part of the fabric, wood or other material bearing the stain, is digested with water containing a few drops of ammonia. When the liquid takes up no more coloring matter, it is filtered, if necessary, and introduced into a narrow little glass trough having parallel sides, which is then placed before the spectroscope; a ray of direct solar light is then caused to traverse the liquid and enter the slit (made quite narrow) of the spectroscope. If the dark bands characteristic of oxyhemoglobin be visible, no doubt can exist as to the nature of the liquid under examination, but if the bands be not seen, a thicker layer of the liquid should be examined in the same manner. This may be effected by pouring the solution into a flat-sided bottle, and placing this before the spectroscope. It has also been recommended to evaporate some of the liquid to dryness in a rather flat watch glass, over sulphuric acid in a vacuum, and to examine the absorption spectrum of the residue.

If no absorption bands be seen, the ammoniacal liquid is acidulated with glacial acetic acid, and agitated with its own volume of ether in a glass-stoppered bottle. If the ether do not separate readily, its separation may be effected by the addition of a few drops of glacial acetic acid. The ethereal liquid, which will have a brownish-red color, is then placed before the slit of the spectroscope, and its absorption spectrum is examined; if hematin be present, the dark bands of hematin in acid solution are perceptible, especially a distinct narrow band in the red (see Fig. 23).

According to Hoppe-Seyler, and Dragendorff, a liquid containing $\frac{1}{10000}$ of oxyhemoglobin, examined under a thickness of one centimetre, gives the absorption bands of oxyhemoglobin distinctly, provided the blood be fresh.

The absorption spectrum of hematin is less sensitive, and shows no bands if the dilution be about $\frac{1}{7000}$. In case the bands of oxyhemoglobin be well marked, it is not necessary to obtain those of hematin; but if the bands be poorly defined or somewhat displaced, the hemoglobin should be converted into hematin, and examined as directed.

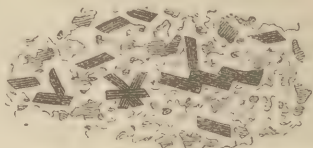
The spectroscopic tests are somewhat uncertain. Should the spot have dried in a position where it was completely exposed to the air, hemoglobin may be detected, even after the lapse of years; but if the coagulation and drying have taken place in the interior folds of a fabric, or other confined position, no characteristic bands may be obtained after two or three weeks.

§ 186. *Hemin crystals*.—The property possessed by hematin of forming characteristic crystals when treated with acetic acid and sodium chloride, has already been studied (§ 89). The crystals are formed when fluid blood is dissolved in glacial acetic acid, and the liquid is evaporated to dryness. A magnifying power of about 300 diameters is necessary for their identification; when prepared from fresh blood, they present the appearances shown in Fig. 57, while when obtained from old stains they resemble Fig. 58. (Naquet.)

Fig. 57.



Fig. 58.



Hoppe-Seyler, Brücke, and Erdmann, have each proposed processes by which the crystals may be produced; in any case, the spots are detached from the objects bearing them, or if upon tissues, they are cut out by the aid of a pair of scissors; from iron or wood, they may be scraped by a sharp knife.

Hoppe-Seyler then recommends that the matter be macerated in a little cold water, and when the latter has removed all of the coloring matter, the liquid is allowed to evaporate spontaneously in a watch-glass. The smallest trace of sodium chloride is added to the dried residue, which has a reddish-brown or brownish color, and the whole is mixed with six or eight drops of glacial acetic acid, by the aid of a slender glass rod. The glass is now gently warmed over a small flame, and the mixture is evaporated to dryness on a water-bath. The residue on the watch-glass is examined under the microscope.

Brücke boils the suspected matter with a little glacial acetic acid, filters the liquid, adds a trace of sodium chloride to the filtrate, and evaporates it to dryness in a watch-glass, at a temperature below 80° . By this means, crystals may sometimes be obtained when Hoppe-Seyler's method fails, as it may when the albuminous matter of the stain has been rendered quite insoluble by washing with hot water, previous to the examination.

Erdmann's process is, however, most satisfactory, and is that which is generally employed. The suspected matter is placed directly upon the microscope slide, a minute particle of sodium chloride added, and the whole is covered with a thin glass cover. A drop of glacial acetic acid is then placed upon the edge of the thin glass cover, so that it may penetrate to the substance by capillarity. After several minutes, the slide is gently warmed by holding it at some distance above a flame, and from time to time it is examined under the microscope. When the liquid becomes sufficiently concentrated, crystals of hemin make their appearance. If no crystals be formed, another drop of acetic acid is placed on the slide, and the alternate warming and examination are repeated, care being taken that the acid is not caused to evaporate too rapidly. Only after several successive trials have failed to yield positive results, can it be decided that no blood is present. The small, colorless cubes of sodium chloride, which may form at the same time, are readily distinguished from the crystals of hemin, but it must be remembered that the smallest perceptible particle of sodium chloride is all that is required for the formation

of hemin crystals, and that a larger quantity may cause the results to be less decisive.

The form and color of the hemin crystals, together with the manner of their formation, are sufficient to characterize them; if further confirmation be desired, it may be obtained by testing the crystals with water, alcohol, and cold acetic acid, in which they are insoluble, and by sodium hydrate, in which they dissolve immediately.

No hemin crystals can be obtained from putrid blood.

The forms of hemin crystals differ as the blood from which they are obtained is derived from different animals, so that it is quite possible to identify the animal by the character of the crystals furnished by its blood.

§ 187. In cases in which no hemoglobin, hematin, or hemin crystals can be detected, iron, albuminoid matters, and ammonia may be detected in stains; but as has already been stated, these tests alone cannot be assumed to prove the presence of blood; and, in the present state of the science, when either of the tests which have been described, especially the hemin test, yields affirmative results, it may be considered that blood was certainly present. The detection of the corpuscles is in itself conclusive evidence.

a) If the spot be wholly or in part soluble in cold water, as it will be should it not have been previously heated or submitted to the action of hot water, the aqueous solution loses its brown or red color when heated, and grayish flakes of coagulated albumen separate. These flakes are soluble in solutions of the alkaline hydrates, and the liquids so obtained act as other alkaline solutions of albuminoid bodies.

b) If the spot be quite insoluble in cold water, it may be dissolved by a rather dilute solution of sodium hydrate, and on the careful addition of either acetic or nitric acid to this solution, a white precipitate of albumen is formed. The alkaline hydrate does not remove the coloring matter of the spot, and the solution will therefore be colorless.

c) If the colored spot which has been treated with sodium hydrate, be extracted with hydrochloric acid, or if a portion of the original spot be detached and treated

with hydrochloric acid, and the liquid be evaporated to dryness, a yellowish residue will be left : if this be dissolved in a few drops of water, the solution will produce a blue color with potassium ferrocyanide, and a red color with potassium sulphocyanide, these reactions being due to the presence of iron in the residue.

d) The guaiac reaction is not characteristic, but may sometimes be serviceable as a confirmatory test. About one cubic centimetre of oil of turpentine which has been exposed to the air for at least several weeks, and has consequently become ozonized, is mixed with an equal volume of a dilute tincture of guaiac; a little of the suspected matter is added, and the mixture is agitated. If any blood be present, the liquid assumes a blue color, and the matter which may be insoluble, is colored dark blue.

SEROUS LIQUIDS.

§ 188. The examination of serous liquids is very similar to that of the serum and plasma of blood, for while the latter fluids differ in some respects from serous exudations, in other respects they are quite analogous. All of the serous liquids contain albumen, and nearly all of them seem to contain at least two distinct albuminoid substances.

They are more or less viscous, sometimes almost colorless and transparent, sometimes highly colored, opalescent or even opaque. Certain of them, such as chyle, lymph, and pus, contain organized constituents, which may be recognized under the microscope.

When globulin, or the fibrinoplastic substance, as well as fibrinogen, is present, they become thick and gelatinous soon after they are removed from the body, and the fibrin which is deposited may be recognized by the characters already described; when these varieties of albumen are not present, the liquids usually remain fluid indefinitely.

The following plan for the qualitative analysis of serous fluids is that proposed by Hoppe-Seyler. After

the presence or absence of the fibrin-forming substances has been ascertained by the spontaneous coagulation, or persistent fluidity of the liquid, a part of the latter is mixed with ten or twenty times its volume of water, a few drops of dilute acetic acid are added, and a current of carbonic acid gas is passed through the liquid. If a cloud be formed, and gradually augment, so that a flocculent precipitate is finally thrown down, the presence of a body analogous to globulin, or an alkaline albuminate, may be considered as demonstrated.

Whether a precipitate be formed or not, the liquid is filtered, and the filtrate heated to boiling; the formation of a coagulum indicates the presence of ordinary albumen, as it exists in serum of blood.

The coagulum produced by carbonic acid gas is then separated from the greater part of the liquid in which it was formed, and divided into two parts; one part is treated with a concentrated solution of sodium chloride; if the coagulum be dissolved, it contains myosin; if it do not dissolve, it may consist of casein. The other part of the coagulum is treated with water containing one-tenth per cent. of hydrochloric acid; if the precipitate dissolve, it consisted of myosin, casein, or fibrin-forming substances.

A part of the serous liquid is then mixed with a few drops of defibrinated blood, obtained by expression from clotted blood, and the whole is allowed to stand twenty-four hours in a warm place. If a gelatinous coagulum be formed during this time, fibrinogen was present in the liquid.

Another portion of the liquid may then be treated with a little of the serous fluid obtained from hydrocele, or from the pericardium of the ox (containing fibrinogen), and if, after a day's standing, the mass assume a gelatinous consistence, or coagulate, the fibrinoplastic substance (globulin) was present.

Paralbumen is precipitated by acetic acid, but is completely redissolved by an excess of that reagent. Its presence may be detected by mixing the liquid under examination with three times its volume of alcohol, collecting the precipitate, and redissolving it in water. If

paralbumen be present, the liquid will become a viscous, jelly-like mass, after a longer or shorter interval, according to the proportion of paralbumen present.

The separation and detection of the other possible constituents of serous liquids, are effected upon precisely the same principles applied for their detection in the blood. Tyrosine, leucine, urea, uric acid, lecithine, biliary matters, cholesterin, and sometimes glucose, are among these substances.

• Quantitative Analysis.

§ 189. ESTIMATION OF WATER AND FIXED MATTERS.—10 or 20 c.c. of the fluid are exactly weighed in a tared porcelain capsule, and evaporated to dryness on a water-bath. The residue is then exposed to a temperature of 100° in the hot-air oven, for several days, or until its weight becomes sensibly constant. The operation may be hastened by raising the temperature to 105 or 110° , after the desiccation has well advanced.

The proportion of extractive matters and of mineral salts may then be estimated by successive extractions with water and alcohol, in a manner analogous to that followed in the analysis of blood (§ 180).

§ 190. ALBUMINOID SUBSTANCES.—It is not often practicable to separate the albuminoid substances for their individual estimation; they are usually estimated together, and this may be most conveniently effected by boiling the liquid with a few drops of acetic acid, and collecting, drying, and weighing the coagulum. The boiling must be continued for some time, and the collected albumen must be washed, first, with a little water, then with boiling alcohol. The results are usually somewhat too low, owing to a small portion of the albumen which escapes coagulation.

The method of Hoppe-Seyler (§ 181) for the estimation of albuminoid substances, fatty matters, salts, etc., in the serum of blood, is equally applicable to other serous liquids.

Special Serous Effusions.

§ 191. The following analyses of some special serous effusions are given by Méhu, principally as the result of observations at the Hôpital Necker.

The examination of the effusion in fifty cases of acute pleurisy in male patients, gave a mean proportion of 63.95 grammes of solid matter per kilogramme. Twelve similar cases in females yielded an average of 64.36 grammes. In thirty-one additional cases (male and female) the average solid matter was 65 grammes. From the history of these cases, Méhu draws the conclusion that a pleural effusion whose specific gravity is above 1018, and which gradually coagulates, indicates an acute pleurisy, whose prognosis is more favorable as the coagulum is more firm. If, on the other hand, the specific gravity is below 1015, the effusion depends upon an obstructed circulation in the heart or in the large vessels; in this case the average solid residue of the liquid falls below 50 grammes per kilo. A large proportion of fibrin generally indicates a favorable termination, but if the liquid be drawn off ten days or two weeks after its effusion, only a small amount of fibrin may be present, the greater part having separated spontaneously in the pleural cavity. When tapping is resorted to several times, the proportion of fibrin augments with each puncture if the disease tend toward recovery, but in the contrary case the proportion of fibrin remains about the same, or diminishes.

The same observer gives as the result of observations in 70 cases of suppurative pleurisy, that notwithstanding the presence of a large proportion of pus, when the residue of solid matter in the filtered effusion, dried at 100°, exceeds 60 grammes per kilo, there is great probability of recovery, while if the solid residue fall below 60 grammes per kilo, the prognosis is extremely grave.

LIQUID OF HYDROCELE.—The effusion in hydrocele bears a great resemblance to serum of blood, but it does not usually contain fibrin. However, fibrinogen is always present, and the liquid coagulates spontaneously on the addition of a small quantity of the fibrinoplastic sub-

stance. The liquid of hydrocele is generally quite limpid, and may be readily filtered. In old effusions cholesterin is usually present, and sometimes in considerable proportion.

Of twenty-six of these liquids examined by Méhu, four contained less than 50 grammes of fixed matter per kilo, twenty-one contained between 50 and 100 grammes, and only one contained more than 100 grammes.

The matter expectorated after thoracentesis is analogous to the pleural effusion, and sometimes even richer in solid matter. This is shown by the following analysis by Méhu of the liquid drawn from the pleural sac of a man 45 years of age, together with that of his expectorations of the day on which the operation was performed.

PLEURAL LIQUID.			EXPECTORATIONS.	
Organic matter	52.44	} 60.06	63.54	} 75.04
Mineral salts .	7.62		11.50	
Water . . .		939.94		924 96
		<hr/> 1000.00		<hr/> 1000.00

The expectoration of a serous liquid containing more than 70 grammes of fixed matter per kilo, should lead to the suspicion of a cyst of the liver, opening into the bronchia, especially if the liquid contain biliary pigment.

P U S.

§ 192. Normal pus is a more or less thick, creamy-looking liquid, having a yellowish or greenish color. It is not tenacious, and flows readily, thus differing from mucus, which it otherwise somewhat resembles. Its specific gravity is about 1030, and its reaction usually neutral or alkaline. By filtration, it may be separated into two portions, one of which forms a limpid serum, while the other consists of solid corpuscles.

The serum of pus contains coagulable albumen, identical with that of the serum of blood, together with another albuminoid body which is precipitated on the addition of dilute acetic acid, and, by its behavior with hydrochloric acid and with sodium chloride, is closely related to myosin and fibrinogen. This substance has been called *pyin*; it may be obtained as a light-yellow solid, by adding very dilute acetic acid to the serum of pus. It is insoluble in acetic acid and in alcohol, but soluble in water. It constitutes but a small proportion of the solid constituents of pus; after its separation, the ordinary albumen may be precipitated by saturating the liquid with sodium or magnesium sulphate, and heating.

The serum of pus also contains mineral salts, cholesterolin, leucine, urea, and glucose. (Hoppe-Seyler.) These substances may be extracted and detected as in the examination of any other serous liquid. The mineral salts consist principally of sodium chloride, and small quantities of alkaline phosphates and sulphates.

The pus corpuscles or leucocytes may be separated from the serum by filtration, and this may be more readily accomplished after the addition of a solution which precipitates them. A dilute solution of sodium sulphate or barium nitrate answers well, and precipitates the corpuscles unaltered. On the contrary, sodium chloride converts them into a jelly-like mass. They contain albuminoid and fatty matters, cholesterolin, lecithine, and mineral salts. By treating them with very dilute hydrochloric acid, thoroughly washing the residue with ether

and alcohol, and then digesting with gastric juice, a residue is obtained which contains substances that have been called nuclein and sulphonuclein. (Miescher.)

Pus may in certain cases be mistaken for mucus, in urine, for example; the distinction may readily be made by adding a little potassium or sodium hydrate, which would completely dissolve mucus, while it renders pus thick and viscid.

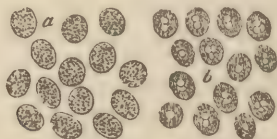
§ 193. BLUE PUS, PYOCYANIN.—Pus sometimes has a blue color, which is attributed to the presence of a peculiar coloring matter called pyocyanin. Fordos gives the following process for the preparation of this substance: The lint, or other matter saturated with the pus, is macerated for twenty-four hours in water containing a little ammonia, after which the greenish liquid is filtered, and agitated with chloroform. The chloroformic solution is decanted and the chloroform distilled off; the residue is then dissolved in a little water, which dissolves the coloring principle and leaves the fatty matters. After filtration, this new solution is agitated with chloroform; the latter is decanted and evaporated to dryness. The pyocyanin so obtained is not quite pure; it is therefore treated with a little hydrochloric acid, with which it forms a red compound, insoluble in chloroform. The hydrochloric acid solution is allowed to evaporate to dryness spontaneously, and the residue is exhausted with chloroform which removes all of the foreign matters, leaving the compound of pyocyanin and hydrochloric acid. This is triturated with barium hydrate under a layer of chloroform, which takes up the pyocyanin set free, and leaves it in blue crystals after spontaneous evaporation of the solution.

Pyocyanin crystallizes in microscopic needles and plates, which are freely soluble in water, alcohol, and chloroform, and less soluble in ether. Its solutions are colored red by acids, and the blue color is restored by the action of alkalis. The color is entirely destroyed by strong acids and by chlorine.

§ 194. Under the microscope, the pus corpuscles appear as small, granular, opaque bodies (Fig. 59 *a*) having different diameters, but generally larger than blood cor-

puscles. They contain one or more variously formed nuclei, which become more apparent when dilute acetic acid is added (Fig. 59 *b*). When treated with liquids

Fig. 59.



Pus cells.

of different densities, pus corpuscles behave in a similar manner to blood corpuscles, contracting in volume when the liquid is more dense than the serum of pus, and swelling, and sometimes bursting, in the contrary case.

The chemical analysis of pus is conducted as that of any other serous liquid. If desired, the estimation of the constituents of the serum and of the suspended matter may be made separately.

EXTRACTS OF THE MUSCULAR TISSUES.

§ 195. The following principles have been found in the liquid obtained by extracting the muscles with cold water: albumen, creatine, creatinine, xanthine, hypoxanthine, caruine, uric acid, glucose, inosite, lactates, salts of the fatty acids, and mineral salts, principally alkaline chlorides and phosphates.

It has been established that the muscular juices are alkaline during life, but as soon as cadaveric rigidity is manifested, or when the muscles are tetanized for a sufficient time, these juices become acid. The changes to which this difference in reaction is due, are not known, but the reaction is independent of the coagulation of the muscular plasma, which may indeed be coagulated without destroying the alkaline reaction, provided the experiment be made before cadaveric rigidity appears.

According to Neubauer, the presence of creatinine in the muscles is not certainly proven, that substance being probably formed by a metamorphosis of the creatine by the processes adopted for its separation. Fresh muscles do not seem to contain glucose, but glycogen, which is converted into glucose either when the muscles are brought into activity, or when the reaction of the juices becomes acid.

§ 196. Processes by which the substances present in extracts of the muscles may be detected, have been described in the first part of this work. The following method may be adopted for the separation and detection of creatine, uric acid, hypoxanthine, xanthine, and inosite:—

The flesh, freed from fat, nerves, and bloodvessels as perfectly as possible, is finely divided, and macerated for about an hour with five or six times its weight of cold water. The aqueous extract is then decanted, and replaced by a smaller quantity of water; after some time this liquid is poured off, and the residue is strongly pressed in a cloth; all of the liquids so obtained are united, and allowed to stand for a short time, after which

any fatty matter that has separated is removed mechanically. The reddish and somewhat clouded liquid is then rapidly heated to the boiling point, allowed to cool, and strained through a cloth, in order to separate the coagulated albuminoid matters. The filtered liquid should be clear, only slightly colored, and will have an acid reaction.

Concentrated solution of barium hydrate is then added until it produces no further turbidity, and the precipitate, which may contain uric acid, xanthine, and hypoxanthine, is separated by filtration and set aside. Carbon dioxide is passed through the filtrate, which is then heated to boiling, in order that all of the barium carbonate may be precipitated. This precipitate is united with that previously obtained, and the filtered liquid is distributed in several small capsules, and concentrated at a low temperature on a water-bath. The pellicles which form on the surface of the liquid during evaporation, are removed and added to the former precipitates.

When the liquid becomes somewhat thick in consistence, it is placed in a warm place, and allowed to evaporate spontaneously. Creatine gradually separates in short colorless needles; these are separated from the mother-liquor by filtration or decantation, washed, first with a little water, then with a small quantity of alcohol, and finally recrystallized in water, as directed in § 57. Another crop of impure crystals may be obtained by the spontaneous evaporation of the mother-liquor.

For the detection of volatile fatty acids, the liquid from which the creatine has deposited is rendered strongly acid by dilute sulphuric acid, and any barium sulphate that is formed is separated by filtration. The filtrate is introduced into a small retort, and distilled at a temperature not above 150° , on a sand-bath. The distilled liquid is examined for the presence of formic, acetic, and butyric acids, as has been directed for the separation of those acids. (§ 9.)

The residue of the distillation is repeatedly agitated with successive portions of ether, and the decanted ethereal solutions are united, and evaporated to a syrupy consistence on a water-bath. The residue is extracted

with alcoholic ether, and the ether driven out by the application of a gentle heat; the alcoholic liquid is mixed with a slight excess of milk of lime, heated to boiling, and filtered hot. On cooling, crystals of calcium lactate are gradually deposited, and are recognized as indicated in § 11.

Any inosite present in the juice would be left in the acid solution remaining after the treatment with ether; this is mixed with boiling alcohol, and any precipitate that may be formed is separated either by decantation, filtration, or both combined. The inosite may then be sought in the filtrate.

Uric acid, if present, is in great part contained in the precipitates produced by baryta-water, and in the pelli-cles removed from the surface of the original solution of creatine; but a portion of it remains in the mother-liquor from the crystallization of the creatine, and may be precipitated by the addition of an excess of alcohol. This precipitate and the baryta residues are united, and treated with glacial acetic acid; after standing one or two days, any uric acid present crystallizes out, together with the xanthine. The uric acid may be recognized by its microscopic appearance, and, if the residue be treated with cold dilute ammonia, the xanthine will be dissolved, while ammonium urate will remain. The murexide test may then be applied to the residue; the ammoniacal solution of xanthine may be precipitated by ammoniacal silver nitrate, and the precipitate identified by the aid of the microscope.

§ 197. The following process was devised by Neubauer, especially for the extraction and separation of xanthine and hypoxanthine; one or two kilogrammes of meat should be employed: The finely divided flesh is thoroughly mixed with about its own weight of water, and heated to 60°, on a water-bath; the mass is then strongly pressed in a cloth, and the residue is mixed with more water, and again pressed. The united liquids are rapidly heated to boiling, and the coagulated albumen is separated by straining through a cloth. The filtrate is allowed to cool, and is then treated with basic lead acetate, as long as a precipitate continues to form, employ-

ing, however, as slight an excess of the lead salt as possible. The precipitate is separated by filtration, and if it be desired to test for uric acid or inosite, is reserved for that purpose, since it will contain those compounds, if present. The clear filtrate is freed from the excess of lead by a stream of hydrogen sulphide, and the solution is filtered from precipitated lead sulphide, and evaporated to a syrupy consistence on a water-bath. It is then allowed to stand for a few days, in order that the creatine may crystallize out. The crystals are separated on a small filter, and repeatedly washed with alcohol. The mother-liquor is united with the alcoholic washings, and the alcohol is expelled by concentration on a water-bath; when the bulk of the liquid is reduced to 100 or 200 c.c., ammonia is added until the reaction is decidedly alkaline, and the solution is precipitated by ammoniacal silver nitrate. The precipitate is thoroughly washed with ammoniacal water, first by decantation, and finally on a filter. It is then boiled with nitric acid of a density of 1.1, until all has dissolved, except a trace of silver chloride which may remain. The clear liquid is poured into a crystallizing dish, and allowed to stand; in about six hours, the compound of hypoxanthine and silver nitrate separates, and is removed and treated with an ammoniacal solution of silver nitrate, to remove free acid, after which it is suspended in boiling water, and decomposed by hydrogen sulphide. After the silver sulphide is separated by filtration, the solution is concentrated and will deposit pure hypoxanthine.

The xanthine remains in the mother-liquor from which the hypoxanthine-silver compound has crystallized. This liquid is mixed with an excess of ammonia, and the precipitate which forms is washed, suspended in boiling water and decomposed by hydrogen sulphide. The filtered liquid deposits xanthine.

JUICES OF THE GLANDULAR ORGANS.

§ 198. The following are the substances which may usually be found in extracts of glandular tissues: albumen, creatine, urea, uric acid, xanthine, hypoxanthine, leucine, tyrosine, cystine, taurine, glucose, inosite, lactic and succinic acids, volatile fatty acids, and mineral salts. Naturally, the constituents of different glands are not the same; biliary acids and pigments may sometimes be found in the liver, and traces of copper may usually be detected in that organ.

The analysis of the glandular organs may be effected by the process indicated for the analysis of muscular tissues. The following process is recommended by Staedler:—

The organs are finely divided by trituration with coarsely powdered glass, and the mass is suspended in cold water, and strained through a cloth. The filtrate is freed from albumen by rapidly heating it to ebullition, and again passing through a cloth. The clear liquid is precipitated by basic lead acetate, the precipitate separated by filtration, and a current of hydrogen sulphide passed through the filtrate. After removing the lead sulphide formed, the liquid is evaporated to a syrupy consistence, and treated with strong, boiling alcohol. The solution is concentrated and allowed to crystallize; leucine and tyrosine, and occasionally taurine, are thus obtained, and gelatin is sometimes left as a residue insoluble in alcohol. The lead precipitate may contain uric acid, xanthine, hypoxanthine, inosite, cystine, and sometimes a little taurine and tyrosine. It is washed, suspended in water, decomposed by hydrogen sulphide, and the filtered liquid is evaporated to crystallization. Processes for the separation and identification of the different compounds have been indicated in the first part of this work.

BONE, TEETH, AND BONY STRUCTURES.

§ 199. The study of the structural formation of bony tissues does not belong to chemistry, and is considered in works on histology. From a chemical point of view, bony tissue consists of two principal constituents, one of which is a mineral framework or skeleton, while the other is cartilaginous and consists of ossein.

The mineral salts which enter into the composition of bone, are, in the order of their preponderance, calcium neutral phosphate, $\text{Ca}^3(\text{P O}^4)^2$; magnesium neutral phosphate, $\text{Mg}^3(\text{P O}^4)^2$; calcium carbonate, and small quantities of calcium fluoride.

In addition to the substances mentioned, bones contain water, fatty and albuminoid matters, alkaline chlorides and sulphates, and a trace of iron; these, however, do not belong to the true bony tissue, but to the bloodvessels which penetrate the bones, to the cell walls of the osseous structures, and to the contents of the medullary canals and canaliculi.

If bones be entirely immersed in very dilute hydrochloric acid for a few days, at a low temperature (15 or 16°), the whole of the mineral matter is dissolved, while the cartilage, or ossein remains, and retains perfectly the form of the bone. In this condition ossein has a yellow color, is transparent, and is flexible and elastic, but it becomes hard, horny, and somewhat brittle, when dried. When ossein is boiled with water, it is entirely converted into gelatin, the rapidity of the change being increased by operating under pressure, thus raising the temperature.

If bones be treated with warm, dilute hydrochloric acid, carbon dioxide is disengaged in considerable quantity, and the bones become fissured, and split into fibrous laminæ.

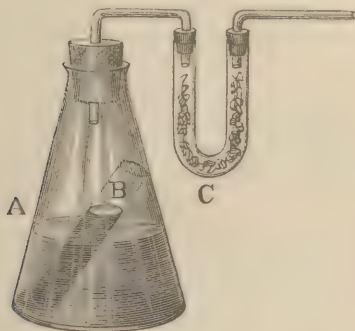
By strongly calcining bone, all of the organic matter is destroyed, and a white, earthy residue, retaining the form of the bone, is obtained, in which the mineral constituents of the bone may be detected by the usual chemical tests.

Quantitative Analysis.

§ 200. In order that the result of the analysis may express the composition of true bony structure, a compact portion of bone should be chosen, and separated from the spongy parts by scraping with a chisel or sharp knife. The periosteum is then carefully removed, as well as all adhering fatty matters; the latter may be completely separated by leaving the bone for some time in contact with ether. The bone is then broken into small fragments, either in a clean iron mortar, or by wrapping it in filter-paper and crushing it by the blows of a hammer. The fragments are inclosed in a clean muslin cloth, and the whole is suspended for several days in a vessel of distilled water, the latter being changed once or twice every twenty-four hours. In this manner all of the soluble salts, which do not form part of the true osseous tissue, are removed. The fragments are now drained, dried, and heated to about 140° , in an air oven, for several hours, after which they may be readily reduced to an impalpable powder. The latter is again heated to about 130° , until its weight, after cooling, becomes constant. It is then ready for analysis.

a) ESTIMATION OF CARBON DIOXIDE.—The simplest

Fig. 60.



and most readily constructed apparatus for the estimation of carbonic acid gas is represented in Figure 60.

Two or three grammes of the dried and pulverized bone are accurately weighed, and introduced into the flask A, which should have a capacity of about 150 cubic centimetres, together with a small quantity of distilled water. A test-tube, B, is then partly filled with hydrochloric acid, and carefully placed in the flask in an upright, or slightly inclined position. The pierced cork of the flask is provided with a chloride of calcium tube, C, destined to prevent the escape of moisture with the carbon dioxide disengaged. The apparatus is now accurately weighed, after which, by inclining the flask, the hydrochloric acid is caused to flow from the test-tube and mingle with the water. An effervescence immediately takes place, due to the decomposition of the calcium carbonate, and carbon dioxide is eliminated. When the effervescence has ceased, the flask is heated on a sand-bath, until the liquid just begins to boil; it is then allowed to cool, and, when its temperature is reduced to that of the surrounding air, is carefully weighed. The loss of weight indicates the amount of carbonic acid gas evolved from the powdered bone. 100 parts of carbon dioxide correspond to 227.27 parts of calcium carbonate.

b) ESTIMATION OF CALCIUM.—Two or three grammes of the powdered and dried bone are carbonized in a platinum or porcelain capsule, at as low a heat as will effect the carbonization; the black mass is exhausted with boiling dilute hydrochloric acid, the excess of acid is expelled by evaporation of the clear, filtered solution thus obtained, and the residue is diluted with water, and an excess of ammonia added. The precipitate formed is dissolved in the smallest possible quantity of acetic acid, which is added to the original liquid, and the solution is precipitated by the addition of ammonium oxalate. The calcium is thrown down as calcium oxalate, while the magnesium remains in solution. After standing twenty-four hours, the precipitate is collected on a filter, and thoroughly washed, the filtrate and washings being retained. The precipitate is dried on the filter, and converted into calcium carbonate by calcination. Since some calcium oxide is formed during the incineration, a few drops of a concentrated solution of ammonium carbonate

are added to the residue, after the crucible has cooled, and the water and excess of ammonium carbonate are expelled by again heating to dull redness. The residue, consisting of calcium carbonate, contains 40 per cent. of calcium, or 56 per cent. of calcium oxide.

c) ESTIMATION OF MAGNESIUM.—The magnesium phosphate remains in the filtrate and wash-water from the calcium oxalate. This is treated with an excess of ammonia, which causes the precipitation of ammonio-magnesium phosphate MgNH_4PO_4 . The mixture is allowed to stand twenty-four hours, in order that the precipitation may be complete, after which the precipitate is collected on a filter, washed with water containing a little ammonia, dried, and converted into magnesium pyrophosphate by strong ignition. 100 parts of magnesium pyrophosphate $\text{Mg}_2\text{P}_2\text{O}_7$, thus obtained, correspond to 36.06 parts of magnesium oxide.

d) ESTIMATION OF PHOSPHORIC ACID.—Part of the phosphoric acid which originally existed as calcium phosphate has been already precipitated in the estimation of magnesium. The remainder exists in the filtrate and wash-water from the ammonio-magnesium phosphate. This is mixed with ammonium chloride and magnesium sulphate, and, after standing twenty-four hours, the precipitated ammonio-magnesium phosphate is collected, washed with ammoniacal water, and ignited as already indicated in the preceding paragraph. 100 parts of magnesium pyrophosphate correspond to 63.96 parts of phosphoric anhydride, P_2O_5 ; the proportion of phosphoric anhydride precipitated in the estimation of magnesium phosphate is calculated from the weight of the magnesium pyrophosphate then obtained, and the quantity so found is added to that calculated from the last estimation.

e) ESTIMATION OF ORGANIC MATTER.—The percentage of organic matter is indicated by the difference between 100 and the sum of the mineral matters found in the bone; this estimation may be controlled by strongly incinerating a small quantity of the powdered and dried bone in a platinum capsule. The residue is then moistened with a few drops of a saturated solution of ammonium carbonate,

and again heated to dull redness. Its weight expresses the quantity of mineral matter, and should closely correspond with the sum of the inorganic constituents estimated separately. If desired, the proportion of fat may be estimated by exhausting a weighed quantity of dried and powdered bone, which has not previously been washed with either alcohol or ether, with strong ether, evaporating the ethereal solution, and weighing the residue, after drying at 100° .

Calculation of the Analysis.

We will suppose that 2.576 grammes of the powdered bone have yielded 0.081 gr. of carbon dioxide (1).

2.651 grammes of powdered bone, treated as in *b*, have given 1.709 gr. of calcium carbonate (2); and from the filtrate after precipitation of calcium oxalate (*c*), 0.071 gr. of magnesium pyrophosphate have been obtained by the addition of ammonia and incineration of the precipitate (3). In addition, the phosphoric acid remaining in the filtrate from the ammonio-magnesium phosphate, has yielded, when treated as in *d*, 1.053 grammes of magnesium pyrophosphate (4).

Since 100 parts of CO_2 correspond to 227.27 parts of calcium carbonate, 100 parts of the bone analyzed contain $\frac{0.081 \times 100}{2.576} = 3.14$ parts of carbon dioxide; or the bone contained $\frac{227.27 \times 3.14}{100} = 7.14$ per cent. of calcium carbonate, which contained $\frac{56 \times 7.14}{100} = 3.99$ parts of calcium oxide.

From (2), since 100 parts of calcium carbonate correspond to 56 parts of calcium oxide, and since 2.651 gr. of bone have been found to yield 1.709 gr. of calcium carbonate, we deduce that 100 parts of the bone analyzed would yield $\frac{1.709 \times 100}{2.651} = 64.46$ parts of calcium carbonate; therefore the bone contains $\frac{56 \times 64.46}{100} = 36.09$ per cent. of calcium oxide.

Since 100 parts of magnesium-pyrophosphate correspond to 36.02 parts of magnesium oxide, and to 63.96 parts of phosphoric anhydride, (3) shows us that 100 parts of bone contained $\frac{0.071 \times 100}{2.651} = 2.67$,

$$\frac{2.67 \times 36.06}{100} = 0.97 \text{ parts of magnesium oxide.}$$

Also, the 2.67 parts of magnesium pyrophosphate correspond to $\frac{2.67 \times 63.96}{100} = 1.71$ parts of phosphoric anhydride (5).

The final determination of phosphoric acid (4) has shown us that 2.651 grammes of bone yield, in addition to the phosphoric acid precipitated in (3), 1.053 gr. of magnesium pyrophosphate. Then

$$\frac{1.053 \times 100}{2.651} = 39.72, \quad \frac{39.72 \times 63.96}{100} = 25.40 \text{ parts of}$$

phosphoric anhydride, plus the 1.71 parts before precipitated, = 27.11 parts, are contained in 100 parts of bone.

We thus far have found—

Calcium carbonate	7.14
Calcium oxide (total)	36.09
Magnesium oxide	0.97
Phosphoric anhydride	27.11
	<hr/>
	67.31

The remaining 32.69 per cent. are considered to consist of fluorine and organic matter.

But part of the phosphoric anhydride existed as magnesium phosphate, the remainder being in the form of calcium phosphate. Since 100 parts of magnesium oxide correspond to 118.33 parts of phosphoric anhydride, and to 218.33 parts of magnesium phosphate,

$$\frac{0.97 \times 118.33}{100} = 1.14 \text{ parts of the phosphoric anhydride}$$

originally existed as magnesium phosphate, of which the

$$\text{bone contained } \frac{0.97 \times 218.33}{100} = 2.11 \text{ per cent.}$$

The remainder of the phosphoric anhydride existed as calcium phosphate. 100 parts of phosphoric anhydride correspond to 118.31 parts of calcium oxide, and to 218.31 parts of calcium phosphate, $\text{Ca}^3(\text{PO}^4)^2$; hence 100 parts of bone contain

$$27.11 - 1.14 = \frac{25.97 \times 218.31}{100} = 56.69 \text{ parts of calcium}$$

phosphate, corresponding to $\frac{25.97 \times 118.31}{100} = 30.72$ parts of calcium oxide.

The total percentage of calcium oxide found was 36.09, of which 3.99 exists as carbonate, and 30.72 as phosphate. The difference between 36.09 and $30.72 + 3.99 = 1.38$, corresponds to the calcium fluoride present in the bone. 100 parts of calcium oxide correspond to 139.46 parts of calcium fluoride, therefore

$$\frac{1.38 \times 139.46}{100} = 1.92 \text{ per cent. of calcium fluoride.}$$

The complete analysis of the mineral matter of the bone has thus yielded for 100 parts of bone—

Calcium carbonate	7.14
Magnesium phosphate	2.11
Calcium phosphate	56.69
Calcium fluoride	1.92
Organic matter (by difference)	32.14
	<hr/>
	100.00

According to Heintz, by whom the preceding method of analysis was proposed, 100 parts of fresh bony tissue from the human femur, gave in two analyses—

	I.	II.
Calcium carbonate	6.36	6.45
Magnesium phosphate	1.23	1.21
Calcium phosphate	60.13	62.46
Calcium fluoride	3.52	2.12
Organic matter	28.76	28.76
	<hr/>	<hr/>
	100.00	100.00

SALIVA.

§ 201. The saliva is the mixed secretion of the sub-maxillary, sub-lingual, and parotid glands, and of the smaller sub-buccal glands. It is a somewhat viscous liquid, slightly bluish, or opalescent in appearance. Its specific gravity varies between 1004 and 1006, and its normal reaction is alkaline. On standing, it deposits a sediment of epithelial matter derived from the mouth and salivary glands.

Normal mixed saliva is essentially an aqueous solution of albumen, mucin, fatty matters, a trace of potassium sulphocyanide, a peculiar ferment called ptyalin, and mineral salts, principally potassium and sodium chlorides, and alkaline and earthy phosphates.

Urea has also been detected in the saliva in apparently normal conditions, and glucose, biliary pigments, lactic acid, and other substances, have been found pathologically. Besides this, certain salts when administered internally rapidly pass from the blood into the saliva, and may easily be detected; among these, the alkaline bromides and iodides, and the salts of mercury, are especially prominent.

Normal saliva yields a flocculent coagulum when boiled, or when treated with nitric acid, alcohol, lead acetate, tannin, mercuric chloride, etc. It is not affected, however, by the addition of either acetic, hydrochloric, or sulphuric acids, or by potassium ferrocyanide, or caustic alkalies.

Ferric salts produce a blood-red color, due to the formation of ferric sulphocyanide.

The ferment of the saliva, ptyalin, has the property of converting starch into glucose, and it may sometimes be of importance to determine whether the saliva is properly active in this respect. For this purpose, a little boiled starch is made into a thin paste with water, and, after the addition of about one-third its volume of the saliva, is maintained at a temperature of about 35° (that of the body). In about fifteen minutes, a portion of the

liquid is tested by Fehling's solution, and the test may be repeated several times, at intervals of fifteen minutes, until satisfactory evidence of the presence or continued absence of glucose is obtained.

After exposure to the air for several days, the saliva loses its property of converting starch into glucose. Concerning ptyalin, to which this property is generally attributed, little is positively known, but it seems to be closely allied to diastase, the peculiar ferment which is formed during the germination of barley and other grains. Its activity is destroyed by the presence of considerable proportions of either acids or alkalies, but is not affected in liquids only slightly acid or alkaline.

Analysis of saliva.—As the chemical principles contained in the saliva are not numerous, the qualitative analysis may be confined to the tests for albuminoid substances, fatty matters, and mineral salts. The latter may be detected in the ash, but bromides, iodides, mercurial salts, etc., which have been administered internally, may be sought for in the saliva without any preparation. Abnormal substances, such as urea, biliary pigment, etc., are detected by the same processes described for the examination of urine and blood.

The quantitative analysis of saliva may be limited to the estimation of water, total fixed matters, extractive matters soluble in alcohol, and mineral salts. The proportion of mucin and epithelial matter may be approximately estimated by evaporating a weighed quantity of the liquid to a very small bulk, adding a few drops of acetic acid, and collecting the precipitated mucin and epithelial cells on a filter which has been dried at 100° and weighed. The mass is then washed with a little water, and the residue dried at 100° , and weighed.

Febrile conditions of the system do not seem to affect the relative composition of the saliva, although the secretion may be diminished or entirely suppressed.

According to Hoppe-Seyler, the saliva secreted in icteric conditions of the system never contains biliary pigments, notwithstanding the contrary assertions of Wright; and in cases of diabetes the saliva is acid, but never contains glucose.

§ 202. *Salivary concretions.*—Calculous concretions are occasionally formed in the salivary glands; they consist principally of calcium carbonate and phosphate, together with an albuminoid substance. Such calculi may be analyzed by triturating them, and treating the powder with dilute hydrochloric acid, which dissolves the mineral salts and leaves the organic matter. The acid solution is evaporated to dryness, the residue heated to redness, and when cool moistened with ammonium carbonate, again heated, and weighed after cooling. The residue consists of the mineral salts. Or, a weighed portion of the powder may be exhausted with boiling water; the insoluble residue is dried on a tared filter and weighed; then incinerated, moistened with ammonium carbonate, again heated and weighed. In this manner the proportion of the substance soluble in water, the proportion of mineral salts, and that of organic matter, may be estimated. The proportions of calcium carbonate and phosphate may be estimated in the ash. The deposits of tartar which frequently form on the teeth, have about the same composition as salivary calculi, and are analyzed in the same manner.

GASTRIC JUICE.

§ 203. Gastric juice, obtained by mechanical irritation of the empty stomach by an inert body, is an almost transparent, and nearly colorless liquid. It is not viscid, and may be easily filtered. It has an acid taste, and a peculiar characteristic odor. Its specific gravity is very little higher than that of water, ranging from 1001 to 1010. Its reaction is strongly acid, and in this respect it differs from the other secretions.

The essential chemical elements of gastric juice are hydrochloric acid, and a nitrogenized organic substance which is soluble in water, and to which the name pepsin has been given; this is the ferment of the gastric juice. There are also present certain mineral salts, notably potassium, sodium, ammonium, calcium, and magnesium chlorides and sulphates, ferrous chloride, and calcium and magnesium phosphates. Under certain conditions, particularly in diseased conditions of the system, lactic, acetic, and butyric acids, have also been detected in the gastric juice; their presence may frequently be demonstrated in vomited matters.

Potassium iodide, potassium sulphocyanide, salts of iron, and glucose, injected into the veins, pass into the gastric juice.

Partially digested albuminoid bodies, called peptones, are nearly always found in gastric juice; they are formed by the action of the juice on ingested matters, or perhaps on the substance of the gastric glands.

The activity of the gastric juice, its power of converting albuminoid bodies into soluble peptones which may be easily digested, is due to the combined action of pepsin and hydrochloric acid. Neither of these substances alone is capable of digesting albumen; when gastric juice is exactly neutralized by an alkali, it becomes inactive upon albuminoid bodies; and while water containing from one-twentieth to one-tenth of one per cent. of hydrochloric acid will dissolve certain albuminoid bodies, the solution so obtained is entirely different from that

which results from the digestion of albumen in a similar liquid containing pepsin. It must be remembered that the gastric juice does not only dissolve albumen, but modifies it (apparently by hydration), converting it into peptone. Thus, white of egg, which does not coagulate in the stomach, being soluble, is not, however, digestible without the aid of an acid, and its complete digestion requires a longer time than that of fibrin, or even of coagulated albumen.

Gastric juice is precipitated by the addition of alcohol; the precipitate is soluble in water, and the solution acquires active digestive properties when treated with a drop or two of hydrochloric acid.

ANALYSIS OF GASTRIC JUICE.

§ 204. The detection of the various constituents of gastric juice may be effected by means which have already been described when treating of these constituents individually.

The proportions of water, solid matter, and mineral salts, are estimated precisely as in the case of serous liquids, the saliva, etc.

Estimation of the Degree of Acidity and of Free Hydrochloric Acid.

The total acidity of the gastric juice is determined by the aid of a decinormal solution of sodium hydrate, the operation being performed as in the estimation of the degree of acidity of the urine.

G. Schmidt first established the fact that the acidity of gastric juice is due to hydrochloric acid, employing the following process:—

A weighed quantity of the previously filtered juice is acidulated with nitric acid, and all of the chlorine is precipitated by adding solution of silver nitrate. The silver chloride formed is collected on a filter, thoroughly washed, first with dilute nitric acid, then with pure water, and finally dried, fused, and weighed. The

weight of the filter-ash being deducted, the proportion of chlorine is calculated from that of the silver chloride.

The filtrate and wash-water are united, freed from excess of silver nitrate by the addition of hydrochloric acid and subsequent filtration, and the filtered liquid is evaporated to dryness and the residue incinerated. The proportions of calcium, magnesium, potassium, sodium, and sulphuric and phosphoric acids contained in the ash, are determined by the usual methods of quantitative analysis.

In another portion of the gastric juice, the ammonia is estimated by the process of Neubauer and Schlössing, as has been described for the urine.

The potassium and sodium found are then calculated as combined with the sulphuric acid; and the other metals are calculated as chlorides and as acid phosphates. The excess of chlorine must be considered to exist as free hydrochloric acid, and the proportion of hydrochloric acid so found corresponds with the degree of acidity of the gastric juice, as determined by the direct acidimetric method.

The process is complicated, and requires perfect familiarity with the practice and calculation of analyses; when these conditions are fulfilled, the results obtained may be regarded as certain.

From the results of analyses by this method, it follows that hydrochloric acid is the normal acid of the gastric juice, and although other acids may be sometimes present, and have been detected by various observers, their presence must be regarded as either accidental or pathological.

BILE.

§ 205. The color of the bile varies from pale-yellow to black ; it is generally viscous, and when agitated froths like a solution of soap. Its specific gravity is subject to great variations, but is usually comprised between 1026 and 1032. It has a peculiar odor, and a persistent bitter taste.

Human bile consists essentially of an aqueous solution of the sodium salts of taurocholic and glycocholic acids, cholesterin, fatty matters, biliary pigments, especially bilirubin, lecithine, and certain mineral salts ; sodium chloride, potassium chloride, sodium phosphate, calcium and magnesium phosphates, and sometimes traces of copper. Mucus from the gall-bladder is also present.

It is probable that the phospho-glyceric acid and neurine, which may be detected in the bile, do not exist there as such, but are formed by the decomposition of lecithine. Bile which has begun to decompose contains cholic acid, produced by the breaking up of the glycocholic and taurocholic acids.

Certain substances appear in the bile under pathological conditions ; they are urea, after extirpation of the kidneys, in cholera, and in Bright's disease ; leucine and tyrosine, in diseases of the liver ; glucose, in diabetes mellitus ; blood, pus, and sometimes albumen.

Normal bile is neutral or slightly alkaline ; it rapidly decomposes in the presence of the mucus it contains, and which seems to act as a ferment ; but if this be precipitated by alcohol and removed, the bile may be preserved indefinitely. When evaporated, it becomes covered with a pellicle, similar to that which forms on the surface of milk under the same conditions.

Bile is miscible in all proportions with water and alcohol, but the latter first precipitates a flocculent deposit of mucus, more or less colored by the pigments present. Alkalies change the color of the bile, but acids produce precipitates. Neutral lead acetate throws down lead glycocholate, and if the liquid be filtered, lead taurocho-

cholate may be precipitated by the addition of tribasic lead acetate to the filtrate.

When bile is agitated with several times its volume of chloroform, it is partly dissolved by the latter liquid, and if the solution be decanted and allowed to evaporate, crystals of bilirubin and cholesterin are deposited.

The extraction and properties of the biliary acids and pigments have already been studied.

Biliary Calculi.

§ 206. Biliary calculi are found in the gall-duct, gall-bladder, and occasionally in the intestines. They are sometimes small, and may then exist in considerable number; sometimes they attain a diameter of two or more centimetres. In shape, they may be nearly round, or irregular and polyhedral. They are usually of a light-brown color, and are greasy to the touch, and so soft that they may be readily crushed.

They consist either of nearly pure cholesterin, of biliary pigments, or of both substances together, sometimes arranged in concentric layers. It is not unusual to meet with gall stones of which the interior is pure cholesterin, while the exterior layers are brown and contain biliary pigment together with some mineral salts.

A calculus consisting of cholesterin presents, on being cut or broken, a brilliant, white, radiated structure.

Pigment calculi also frequently show radiated structures, but their color is variable, the same stone sometimes having layers of different colors. The pigments are not deposited in the free state in calculi, but are combined with earthy bases, and accompanied by calcium and magnesium carbonates.

§ 207. ANALYSIS.—A portion of the calculus is pulverized, weighed, and dried at 100 or 110°, in an air-oven, until its weight becomes constant. The loss of weight indicates the proportion of water. The dried powder is then exhausted with a small quantity of boiling water, which removes any bile that might be present; its quantity, usually very small, is given by the weight of the residue left after the evaporation of the aqueous solu-

tion. The powder is again dried, and exhausted with a mixture of equal volumes of strong alcohol and ether. The ethereal extract is evaporated, and the residue, consisting of cholesterin, is dried at 100° , and weighed.

The new residue, from which all cholesterin has been removed, is dried, weighed, and treated with dilute hydrochloric acid, which dissolves out the earthy phosphates and carbonates, and sets free the coloring matters. The acid solution, separated by filtration, may be examined, if desired, for the metals present. The loss of weight of the dried residue of the hydrochloric acid extraction, indicates the proportion of mineral salts present, while the residue itself represents the biliary pigments and mucus. If it be desired to separate the pigments, the residue is exhausted with chloroform, the liquid is decanted, and the chloroform separated by distillation: the residue of the distillation is treated with a mixture of absolute alcohol and ether, which leaves the bilirubin unchanged, and dissolves bilifuscin. The residue remaining after the extraction by chloroform, contains biliprasin, and yields it to alcohol. All of these solutions leave their respective pigments, in a more or less pure state, when they are evaporated to dryness.

The residue of the last extraction consists of mucus, possibly albuminoid matters, and bile pigments which have undergone alteration and become insoluble.

The hydrochloric acid solution obtained should be evaporated to dryness, the residue calcined, and then dissolved in very dilute hydrochloric acid. If hydrogen sulphide be passed through this solution, a black precipitate is often formed, and may be shown by further examination to consist of copper sulphide. As before mentioned, traces of copper have occasionally been detected in bile.

MILK.

§ 208. Milk is essentially an aqueous solution of lactose, together with certain mineral salts, holding in suspension very small fatty particles and, either suspended or in solution, an albuminoid body known as casein. It has a specific gravity of about 1030, subject to considerable variation. Its reaction is generally alkaline shortly after its secretion, but gradually changes to acid by the formation of lactic acid. Sometimes, however, milk is acid at the moment it is drawn; this is said to be usually the case with asses' milk, and frequently with cow's milk. Normal woman's milk is always alkaline.

Milk is coagulated by most acids, mineral and vegetable, especially by the aid of heat. Milk which has been saturated with boric acid may be preserved indefinitely in the cold, and will not coagulate; but it at once coagulates when heated. Some salts, such as zinc chloride and calcium sulphate, have the property of coagulating milk. Lactic acid alone does not coagulate milk in the cold, unless it be present in considerable quantity, but acetic acid soon produces the coagulation, especially when aided by heat. Hence, milk may become sour by the natural formation of lactic acid, but does not usually thicken until acetic acid makes its appearance in the liquid. Rennet, which is prepared from the fourth stomach of the calf, coagulates milk with remarkable facility.

When allowed to stand for several hours, the fatty globules which are suspended in milk gradually rise to the surface, and constitute cream. But the cream does not consist wholly of fat globules, nor does it contain all the fat of the milk. The opaline or bluish layer immediately below the cream, contains the greater part of the casein, lactose, and salts.

The composition of cream is subject to as great variations as that of the milk from which it is derived. The following are the results of six analyses of cream, by Mr. Wanklyn of London.

	I.	II.	III.	IV.	V.	VI.
Water	72.2	71.2	66.36	60.17	53.62	50.0
Fat	19.0	14.1	18.87	33.02	38.17	43.9
Casein, lactose, salts	8.8	14.7	14.77	6.81	8.21	6.1

The composition of milk varies greatly, not only in different animals of the same species, but in the same individual, changing with the age, health, with the time of the day at which the milk is drawn, and even with the stage of the milking: the first portions of milk drawn are usually much more aqueous than the last.

The substances of which the proportions should be estimated in a quantitative analysis of milk, are water and fixed matters, mineral salts, fat (butter), casein, and lactose. The analysis should also lead to the detection of adulterations, should such be suspected.

DENSITY.—The density of normal cow's milk is about 1030, but may vary from 1018 to 1040. However, if the animals be in good condition, the density of unskimmed milk should not fall below 1029; while should the cream have been removed, the density should be 1033. The specific gravity of natural milk may be stated to be comprised between 1029 and 1034. Any considerable variation from these limits should give rise to the suspicion of adulteration. The specific gravity is determined by means of a hydrometer, and the temperature of the milk at the time of the observation should be 15°. Should it be higher or lower than this, one degree of the hydrometer or lactometer is added or subtracted for every 5° in temperature above or below 15°.

Chemical Analysis.

§ 209. Various methods have been devised for the chemical analysis of milk. In principle they are alike, but the modes of operation differ in some respects.

PROPORTION OF WATER AND OF FIXED SUBSTANCES.—The complete evaporation of milk and analogous fluids requires care and patience. The liquid cannot be boiled, for as it becomes thick and concentrated, portions would be projected from the vessel, thus occasioning loss of substance and erroneous results. The desiccation may

be accomplished either in a vacuum, or under a bell-jar over sulphuric acid; but the time required by such a method excludes it from practical application in the majority of cases. No other resource is then left than the hot-air oven.

10 c.c. of the milk are weighed in a small capsule, preferably of platinum, and exposed to a temperature of 100° in an air oven, until the weight of the residue becomes sensibly constant. The capsule is then allowed to cool in a desiccator, and should be covered during the final weighing, since the residue is quite hygroscopic.

When milk is evaporated, a tenacious film forms on its surface, and is re-formed as often as it is removed. The evaporation and drying may be much facilitated by breaking up this film, from time to time, with a small glass rod, or platinum spatula, which, when used, should always be weighed with the capsule. The results given by this method are quite satisfactory, although the residue has a yellow or brownish color, due to the action of the heat on the lactose and fatty matters of the milk.

§ 210. PROPORTION OF CREAM.—The proportion of cream is estimated by allowing about 200 c.c. of the milk to stand for twenty-four hours in a cylindrical jar about 42 millimetres in diameter and 16 centimetres high. The jar is graduated in one-hundredths, and is filled with milk to the 0th point. In about twenty-four hours, the depth of the layer of cream is read off, and the number of divisions it occupies, indicates its centesimal proportion. Normal cow's milk thus treated shows between ten and fourteen per cent. of cream.

Of course such a method is only approximate, and of no real value from a scientific point of view. As has been seen, cream varies greatly in composition, and it can hardly be satisfactory to determine the proportion of a matter whose composition is uncertain. However, the method may be useful in cases where it is suspected that part of the cream has been fraudulently removed.

§ 211. CASEIN.—As has already been mentioned (§ 96), casein appears to be an alkaline albuminate; it is precipitated from milk by the addition of acids, or of certain neutral salts. The recent researches of J. Leh-

mann seem to show that it does not exist in solution in milk, but in a state of suspension; thus when milk diluted with an equal volume of water is poured upon a porous tile, the aqueous portion is absorbed, while the casein and fat remain upon the surface, forming a tenacious film; Lehmann has applied this fact to the estimation of casein, as will be indicated farther on.

Millon and Commaille's Method of Analysis.

§ 212. *a*) The proportion of water and fixed matter is determined precisely as already indicated, by evaporating a known quantity of the milk to dryness in a platinum capsule, either in an air-oven at $100-110^{\circ}$, or in a vacuum over sulphuric acid, until the weight of the residue becomes sensibly constant.

b) CASEIN, FAT, AND ALBUMEN.—20 c.c. of the milk, previously well agitated to insure homogeneity, are diluted to 400 c.c. with distilled water. Very dilute acetic acid is added, drop by drop, to the mixture, until a flocculent precipitate appears, and a current of washed carbon dioxide is then passed through the liquid for a quarter or half an hour. After standing for about twelve hours, the precipitate is collected upon a filter, which has been dried at 100° and weighed, and washed with distilled water. It is then dried at 100° , and weighed. The weight of the precipitate multiplied by 5, gives the weight of the casein and butter in 100 c.c. of milk.

The quantity of albumen is determined by boiling the filtrate, together with the washings from the casein, for a short time, collecting the coagulated albumen upon a tared filter, washing with water, and drying it at 100° until its weight becomes constant.

c) LACTOSE.—The lactose is contained in the last filtrate and washings. These are united, the volume of the mixture is exactly measured, and a 50 c.c. burette is filled to zero with the liquid.

20 c.c. of Fehling's solution (see §§ 23 and 158) are then diluted to 100 c.c. with distilled water, and heated to boiling. As soon as the liquid is in full ebullition,

the solution to be analyzed is slowly added until all of the copper is reduced. The operation is conducted precisely as has been described for the estimation of glucose in urine; however, while 10 c. c. of Fehling's solution would be reduced by 50 milligrammes of glucose, the same quantity of the cupric solution will require 67 milligrammes of lactose. Hence the 20 c. c. employed correspond to 134 milligrammes of lactose.

Suppose that the total volume of the filtrate and wash-water obtained after the coagulation of the albumen be 550 c. c., and that 82 c. c. of this be required for the reduction of 20 c. c. of Fehling's solution; these 82 c. c. must have contained 134 milligrammes of lactose; hence—

$$\frac{134 \times 550}{82} = x, \text{ whence } x = 898 \text{ milligrammes, the}$$

quantity of lactose contained in 550 c. c. Since the latter correspond to 20 c. c. of milk, 100 c. c. of milk analyzed contain $898 \times 5 = 4.490$ grammes of lactose.

d) BUTTER.—20 c. c. of milk are well mixed with about an equal volume of a not too concentrated solution of sodium hydrate, and the mixture is agitated with 60 or 100 c. c. of ether. After the ether has separated, it is decanted into a tared vessel, and the still milky aqueous liquid is agitated with a fresh portion of ether, which is then decanted and added to the first portion. The treatment with ether is continued until a drop of the ethereal extract leaves no fatty residue when allowed to evaporate upon a watch-glass. The united ethereal solutions are then evaporated at a gentle heat, and finally dried in an air-oven at 110° , and weighed. The weight of the residue multiplied by 5, gives the quantity of butter in 100 c. c. of the milk. By subtracting this weight from that of the casein and butter, as first found, the quantity of casein is estimated.

e) MINERAL SALTS.—The residue obtained in *a* is cautiously heated until it is completely carbonized; if it be heated too rapidly, a portion will be projected from the capsule by the swelling of the mass. The charcoal is then exhausted with the smallest possible quantity of boiling water, and the liquid is filtered through a filter of which the weight of the ash is known. The capsule

and residue are washed with boiling water, which is also passed through the filter. The filtrate is evaporated to dryness in a small tared capsule, and the residue is heated to incipient redness. Its weight is that of the soluble mineral salts. The filter and its carbonaceous contents are ignited in a platinum capsule or crucible, until the carbon is completely consumed. The weight of the residue, less that of the filter ash, is the weight of the insoluble salts.

It may be objected to the process of Millon and Commaille, that it estimates as albumen a portion of the casein which is not entirely precipitated except under the influence of heat. Indeed, if the whey obtained by the action of rennet on milk be saturated with sodium chloride, and then mixed with five or six times its volume of alcohol, no precipitate is formed; under the same circumstances, a dilute solution of albumen yields an abundant coagulum. Doyère, Millon, and others have maintained the presence of albumen in milk, because after the casein has been separated by the addition of acetic acid, a coagulum may yet be obtained on heating the clear liquid. This only shows, however, that all of the casein is not precipitated by acetic acid alone without the aid of heat. Hence it would probably be more exact to boil the liquid after the addition of acetic acid, and to estimate the entire precipitate as casein, the filtrate being used for the estimation of lactose, as before.

Method of Chevalier and O. Henry.

§ 213. A measured volume of the milk is boiled, and treated while boiling with a little acetic acid diluted with twice its volume of water. The precipitate is collected on a filter, washed with water, and then exhausted with ether. The ethereal solution is evaporated to dryness, and the residue gives the weight of butter; the casein remains on the filter, together with insoluble salts; it is dried at 100° , and weighed. It is then incinerated with the filter, and the proportion of insoluble salts thus found is deducted from the weight of the precipitate, and the quantity of casein is so ascertained. The aqueous fil-

trate and washings contain the lactose and soluble salts; the former is estimated by submitting a portion of the liquid to volumetric analysis by means of Fehling's solution. The soluble salts are determined by evaporating another portion of the liquid to dryness, lightly incinerating, and weighing the residue.

Baumhauer's Method.

§ 214. A number of glass rings about 4 centimetres in diameter are made from slender glass rods, and two short straight pieces of rod are, at angles of about 60° , attached to the extremities of one diameter of each ring, one above, the other below, so that the ring will rest immovable in a horizontal position in a funnel. A circular filter 10 or 12 cm. in diameter is folded, opened into a cone, and placed in a ring. The filter is then filled with pure, dry, quartz sand, and may be supported in the ring without coming into contact with the sides of the funnel. The upper straight glass rod attached to the ring serves as a handle, by which the ring, with its filter filled with sand, may be removed from the funnel, and supported in the mouth of a small beaker, and weighed. This arrangement is more easily made than that originally employed by Baumhauer, which is represented in Figure 61.

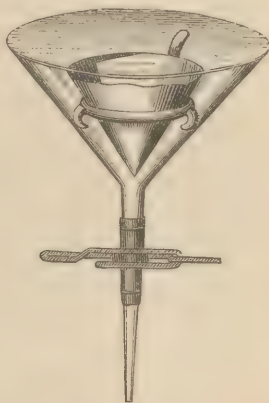
As many rings are necessary as there are analyses to be made; each should be supported in a funnel of which the beak is closed by a gum tube and spring clip, the top being ground, and covered with a glass plate (Fig. 62). A filter so arranged, and filled with sand, is dried for half an hour at 100° , and weighed, together with its ring support. 10 c.c. of the milk to be analyzed are then slowly and carefully distributed over the sand, so that all of the fluid may be absorbed without wetting the paper. The whole is then transferred to an air oven heated to 105° , and dried until the weight of the system becomes sensibly constant. The difference between this weighing and the first, gives the sum of the solid constituents of the 10 c.c. of milk. Sand cools slowly, so that sufficient time must be allowed for the filter to cool

before weighing. The temperature of the air oven should be kept below 100° for four or five hours after the filters are first introduced, and then raised to 105° for another hour or two.

Fig. 61.



Fig. 62.



The filter with its contents is placed in one of the funnels, which is then filled with anhydrous ether, and covered for half an hour. After that time the ether is drawn off by opening the spring clip, and the operation is repeated twice. The filters are finally washed twice, by pouring a little ether on them and allowing it to run through directly, and are then placed in the air-oven and dried. Each filter requires about 100 c.c. of ether. The difference between the weight of the filter, after treatment with ether, and the previous weighing, gives the proportion of butter present in 10 c.c. of the milk. For the estimation of lactose, the proceeding is the same as that indicated for butter, except that warm water, slightly acidulated with acetic acid, is substituted for the ether, and the filtrate is received in a 100 c.c. flask. About 90 c.c. of warm water should be used, in successive portions, and, when this has run through, the filtrate is cooled to 15° , diluted to 100 c.c., and the amount of lactose present is estimated by Fehling's solution.

The filter and contents are again dried, cooled, and weighed, the difference between this weighing and the weight of the filter and sand, before the addition of the milk, representing the quantity of casein and of insoluble salts. The difference between the loss of weight after treatment with water, and the quantity of lactose found, represents the soluble salts. The total quantity of mineral salts, the remaining factor necessary for the estimation of casein and insoluble salts, is found by incinerating 10 c.c. of the milk in a platinum crucible, and weighing the ash.

The method is very convenient when a number of analyses are to be made simultaneously. The results given by it are quite concordant, and may be regarded as satisfactory. It is not, however, absolutely accurate, owing to the slight solubility of casein in water. It is advantageous in the analysis of woman's milk, since only 20 c.c. of milk are required for the complete analysis.

§ 215. Partial Analysis of Milk.

a) VOLUMETRIC ESTIMATION OF LACTOSE.

10 c.c. of Fehling's solution are diluted with 30 c.c. of water, and heated to boiling in a flask or capsule. The milk to be analyzed is diluted with three times its volume of water, and added to the cupric solution directly from a burette. Or the milk may be coagulated by heating it with a few drops of acetic acid, and the clear, filtered whey may be diluted with three volumes of water, and used for the estimation of lactose.

b) RAPID APPROXIMATE ESTIMATION OF BUTTER.

MARCHAND'S PROCESS.—This method of approximate analysis depends upon the fact that the fats are easily soluble in ether, and not very soluble in a mixture of equal volumes of alcohol and ether.

The apparatus devised by Marchand, and called a *lactobutyrometer*, consists of a glass tube about 10 millimetres in internal diameter, and 30 centimetres long, closed at one end. The body of the tube is divided into three

portions of equal capacity (Fig. 63). The tube is filled with milk up to the lower division, marked L; ether is then poured in up to E, and alcohol is finally added up to the line A. The space E A is divided into ten equal parts, and the upper three divisions are marked on the glass and subdivided into tenths, which are called the degrees of the lacto-butyrometer. The subdivisions, equal to the degrees, are also extended above the line A. Each division or degree is equal to one one-hundredth of the space A E, or to one three-hundredth of the total capacity of the body of the tube.

By Marchand's original process, the milk, rendered homogeneous by agitation, is poured in up to the line L, made alkaline by a few drops of sodium hydrate, and the ether and alcohol are then added. C. Méhu has modified the process by suppressing the use of sodium hydrate, and employing an alcoholic solution of boric acid. The modification appears to be an improvement, and the manipulation is then conducted as follows:—

The tube is filled up to L with the milk, and then up to E with dry ether. It is then closed by means of an ordinary close-fitting cork, and agitated until the liquids are thoroughly mixed. Then, without waiting for the separation of the fluids, or paying attention to the contraction in volume, alcohol saturated with boric acid is poured in up to the line A. The tube is then again closed, and vigorously agitated; the precipitated casein breaks up into fine flakes, and readily subsides to the bottom of the tube, only slightly retarding the separation of the butter. The still closed tube is then placed in a vertical position in a water bath heated to 40°.

Fig. 63.



Marchand's Lacto-butyrometer.

The butter gradually rises to the surface, and when its volume ceases to increase further, the number of degrees it occupies is read off.

The operation is precisely the same if the boric acid be omitted; a few drops of sodium hydrate are then added to the milk before pouring in the ether, but, although the butter sometimes separates more quickly under these circumstances, the result is not always satisfactory.

Marchand states that at a temperature of 40° , milk must contain 12.60 grammes of butter per kilogramme in order to saturate with fatty matter the mixture of alcohol, ether, and milk. Hence any butter in excess of 12.60 grammes per kilo, will be set free. The weight of this butter, x , is found by the formula $x = 12.60 \text{ gr.} + n \times 2.33 \text{ gr.}$, n representing the number of degrees of the lacto-butyrometer occupied by the butter. While this method cannot be considered as exact, it is closely approximate, and is of special value in the examination of the quality of commercial milk.

Marchand states that unskimmed milk treated by this method shows an average of 36.34 grammes of butter per kilogramme. The minimum quantity of butter allowed in the milk accepted by the hospitals of Paris, is fixed by law at 30 grammes per kilogramme, and good unskimmed milk should contain at least that much.

ESTIMATION OF CASEIN AND BUTTER.

According to the experiments of J. Lehmann, casein does not exist in a state of solution in milk. When milk diluted with an equal volume of water is spread on the surface of a porous tile, the casein and butter remain on the surface of the tile, while the whey, holding in solution the lactose, the soluble mineral salts, etc., is absorbed. After standing for one or two hours, the film of casein and butter may be perfectly detached by the aid of a thin spatula, dried at 105° for two hours, and weighed. The separation of the butter and casein is easily effected by ether. The casein is then weighed separately, burned, and the weight of the ash, consisting of mineral salts, is deducted from the weight first found.

The amount of butter may be determined by evaporating the ethereal solution and weighing the residue, or by the difference between the total weight of casein and butter, and that of the casein after extraction by ether. The results published by Lehmann are quite satisfactory.

§ 216. The mineral salts which are obtained as ash after the incineration of milk, may be submitted to quantitative analysis. One litre of cow's milk yielded to E. Marchand, 7.28 grammes of ash, and Filhol and Joly obtained 5.98 grammes of ash from one litre of woman's milk; these ashes were constituted as follows:—

	Cow's milk.	Woman's milk.
Potassium chloride,	0.994 grammes.	0.41 grammes.
Sodium chloride,	0.458 “	1.35 “
Potassium phosphate,	0.063 “	{ sodium phosphate traces.
Calcium phosphate,	3.458 “	3.95 grammes.
Magnesium phosphate,	0.657 “	0.27 “
Iron phosphate,	0.248 “	traces.
Potassium sulphate,	0.703 “	
Potassium silicate,	0.018 “	traces of CaF_2 .
Sodium carbonate,	0.671 “	traces.

The following tables show the results of the analysis of cow's milk by different observers and in different countries, the proportions given being calculated for one kilogramme of milk.

	POGGIALE.	COM-MAILLE, Marseilles	QUE-VENNE, Paris.	CAME-RON, Scotland.	COM-MAILLE, Algeria.	GORUP-BESANEZ, Holland.
Water,	862.80	878.03	872.	870.	853.85	839.72
Solid matter,	137.20	121.97	128.	130.	146.15	160.28
Casein,	38	31.32	35.70	41.	35.37	34.87
Albumen,	...	19.08	16.70	7.32
Butter,	43.80	30.10	33.80	40.	43.54	68.46
Lactose,	52.70	35.34	58.50	42.80	43.59	43.50
Mineral salts,	2.70	6.13	...	6.20	6.65	6.14

The following results are calculated for one litre of milk:—

	MARCHAND, Normandy.	WANKLYN, London. Country-fed cow.	WANKLYN, London. City fed cow.	WANKLYN, London. Alderney cow.
Water . . .	910.55	900.9	884.3	898.8
Solid matter . .	121.35	128.1	144.7	130.2
Casein . . .	18.45	41.6	51.6	47.5
Albumen . . .	5.37
Butter . . .	38.40	31.6	41.2	33.1
Lactose . . .	51.85	47.6	44.3	42.4
Mineral salts .	7.28	7.3	7.6	7.2

Comparison of the milk of different animals, proportions in one kilogramme.

	QUEVENNE. Goat.	COMMAILLE. Ewe.	CAMERON. Mare.	CAMERON. Sow.
Water . .	878.40	831.15	903.10	817.60
Solid matter	121.60	168.85	96.90	182.40
Casein . .	27.60	41.85	19.55
Albumen .	6.50	19.09	61.80
Butter . .	30.20	53.75	10.55	58.30
Lactose .	57.30	44.94	62.83	53.35
Mineral salts	9.22	3.97	8.95

The following table shows the composition of 100 parts of woman's milk, from the same woman under different conditions, as analyzed by Simon, and the general mean of many analyses by different observers.

	Density	Solid matter.	Casein.	Butter.	Lactose	Mineral salts.
1 mo. after confinement	1.030	11.62	1.96	3.14	5.76	0.166
45 days " "	1.030	11.64	2.2	2.64	5.2	0.178
3 mos. " "	1.032	13.4	4.52	2.74	3.92	0.287
Suffering from hunger	1.034	8.6	3.55	0.8	3.95	0.24
General average	1.0315	12.3	1.9	4.5	5.3	0.18

The following table shows at a glance the average composition of different milks, the proportions being calculated for 100 parts by weight.

	Water.	Solid matter.	Casein.	Butter.	Lactose	Salts.
Woman's milk	87.7	12.3	1.9	4.50	5.3	0.16 to 0.45
Cow's milk	86.5	13.5	3.6	4.05	5.5	0.30 to 0.90
Ass's "	90.7	9.3	1.7	1.55	5.8	0.5
Goat's "	87.6	12.4	3.7	4.20	4.0	0.56
Mare's "	89.0	11.0	2.7	2.50	5.5	0.5
Ewe's "	82.0	18.0	6.1	5.33	4.2	0.7

Examination of Commercial Milk—Detection of Adulterations.

§ 217. Among the frauds practised in the sale of milk are the following:—

The sale of skimmed milk represented as unskimmed.

The dilution of good milk with water or skimmed milk.

The addition of sodium acid carbonate to prevent the coagulation of the milk.

The addition of starch, flour, or chalk.

The density of milk is often considered as a sufficient criterion of its purity, and if the average amount of cream be present, the criterion is a safe one. As has been mentioned, good unskimmed milk should have a density of about 1030; if it be diluted with water, this density will of course be lowered, but if the cream be removed, it will again be raised, so that a milk of normal specific gravity may be prepared by removing the cream from good milk, and then diluting it with water until the specific gravity is diminished to 1030.

It is hence necessary to combine the indication afforded by the specific gravity with an estimation of the amount of butter present, as found by the lacto-butyrometer (§ 215 b).

After determining whether the milk has or has not been skimmed, the *lacto-densimeter* of Quevenne affords a means of approximately determining the amount of water that has been added to the milk, in case it has been diluted.

The lacto-densimeter consists of an hydrometer, in which only the last two figures of the specific gravity, referred to water as 1000°, are given.

On one side of the scale are marked the points to which the instrument should sink in pure unskimmed milk, and the approximate proportions of water which have been added, should it sink lower than in pure milk. Similar graduations on the other side of the scale refer to skimmed milk.

An ordinary hydrometer may be employed instead of the lacto-densimeter, and the following table will indicate

the proportion of water which has been added to the milk; only the last two figures of the specific gravity (water=1000) are given:—

Water added.	Specific gravity of pure milk.	Specific gravity of skimmed milk.
0	33 to 29	36.5 to 32.5
$\frac{1}{10}$	29 to 26	32.5 to 29
$\frac{2}{10}$	26 to 23	29 to 26
$\frac{3}{10}$	23 to 20	26 to 23
$\frac{4}{10}$	20 to 17	23 to 19
$\frac{5}{10}$	17 to 14	19 to 16

The lacto-densimeter is graduated at a temperature of 15°, for which the above table is also arranged. If the temperature of the milk at the moment of observation be above or below that point, the degree found must be corrected by adding or subtracting the error indicated in the following table.

Degree of lacto- densi- meter.	Pure milk.				Skimmed milk.			
	Temperature.				Temperature.			
	5°	10°	20°	25°	5°	10°	20°	25°
15	—0.9	—0.6	+0.8	+1.8				
20	1.1	0.7	0.9	1.9	—0.7	—0.5	+0.8	+1.7
22	1.2	0.7	1.	2.1	0.7	0.5	0.8	1.7
24	1.2	0.7	1.	2.1	0.9	0.6	0.8	1.7
26	1.3	0.8	1.1	2.2	1.	0.7	0.8	1.8
28	1.4	0.9	1.2	2.4	1.	0.7	0.9	1.9
30	1.6	1.	1.2	2.5	1.1	0.7	0.9	1.9
32	1.7	1.	1.3	2.7	1.1	0.7	1.	2.1
34	1.9	1.1	1.3	2.8	1.2	0.8	1.	2.2

Thus, if the lacto-densimeter sink to 28 in a sample of skimmed milk under examination at 20°, the correct density of the milk, reduced to 15°, will be 1028.9.

Sodium carbonate is sometimes added to milk for the purpose of preventing its coagulation by the formation of lactic acid. This adulteration is not easily detected, since normal milk contains traces of carbonates. About 50 c.c. of the milk should be evaporated to dryness, the residue heated to redness, and then exhausted with water. The aqueous solution is evaporated to dryness, and the dry residue treated with a little hydrochloric acid. An

abundant disengagement of carbon dioxide indicates that an abnormal proportion of a carbonate is present. According to Marchand's analysis, 50 c. c. of normal cow's milk contain about 33 milligrammes of sodium carbonate; the amount of carbon dioxide eliminated from the residue of the aqueous extraction of the ash of 50 c. c. of the milk under examination, might, therefore, be compared with that disengaged from 33 milligrammes of sodium carbonate.

Starch is detected by the aid of the microscope, and by the blue color it assumes when treated with iodine. The starch granules are larger than the milk globules, and are oval (Fig. 64). When examined by polarized

Fig. 64.



Starch granules.

light, each granule appears marked with a dark cross. Before testing with iodine, the milk should first be boiled and allowed to cool; a few drops of tincture of iodine are then added, and, if starch be present, the liquid will assume a dark blue color.

If milk be adulterated with chalk, the latter will be deposited on the bottom of a vessel in which the milk is allowed to stand, and may be recognized by its solubility with effervescence in hydrochloric acid; the solution so obtained responds to the usual tests for calcium salts.

Diseased Milk.

§ 218. Like other secretions, milk undergoes alterations in composition in diseased states of the system. The alteration may consist in an increased or diminished quantity of the normal constituents, or abnormal sub-

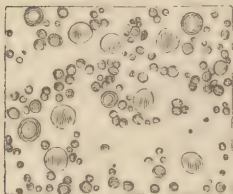
stances may make their appearance. The more usual pathological elements found in milk, are urea, hemoglobin, blood, and pus.

Urea is detected as described in section 166.

The presence of hemoglobin, or of blood globules, is revealed by the altered color of the liquid, and by a microscopical examination. Figure 65 represents the difference between the appearance of blood globules and that of milk globules.

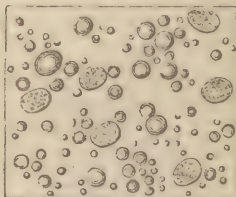
Small quantities of pus may entirely escape detection, but a notable proportion, such as would be present in case of a large abscess in the mammary gland, may be

Fig. 65.



Milk globules and blood cells.

Fig. 66.



Milk globules and pus cells.

detected by the aid of the microscope. Pus cells are larger than milk globules, and as the latter present neither nucleus nor cell wall, both of which are possessed by leucocytes, the distinction is not difficult when a sufficient number of pus cells are present (Fig. 66).

Spots of a blue color sometimes make their appearance on the surface of milk. They appear to be occasioned by a species of fungus.

Colostrum.

§ 219. The liquid secreted by the mammary gland during the first few days after confinement does not present the characters of normal milk, and is called colostrum. It is a viscous, yellowish liquid, having a specific gravity higher than that of milk, generally comprised between 1040 and 1060.

It contains less lactose than milk, and little or no

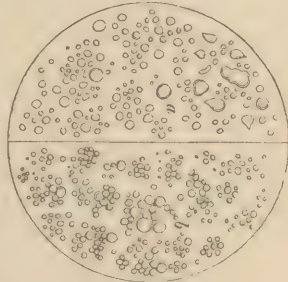
casein, the latter body being replaced by albumen. Consequently, when colostrum is boiled, it coagulates, the albumen becoming insoluble. As the secretion acquires the characters of normal milk, the albumen gradually disappears, and the proportion of casein correspondingly increases.

In addition to the fat globules which are peculiar to milk, colostrum contains large granular corpuscles, having a yellowish color. Milk globules are nearly spherical, and highly refractive; their mean diameter varies from about $\frac{1}{1000}$ to

$\frac{9}{1000}$ of a millimetre: it is uncertain whether they are surrounded by an albuminous envelop, or whether they are merely globules of fat held in suspension (Fig. 67).

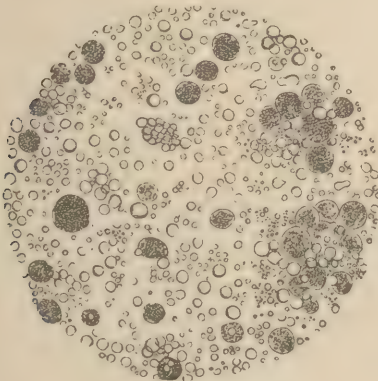
Colostrum globules appear to be agglomerated fat globules (Fig. 68). They vary in diameter from $\frac{1}{1000}$ to

Fig. 67.



Milk globules.

Fig. 68.



Colostrum globules.

to $\frac{56}{1000}$ of a millimetre. They are frequently found in normal woman's milk, but not often in that of the cow

and other animals. However, in certain diseases, cow's milk bears a close resemblance to colostrum.

The following is the result of the analysis by Simon of the colostrum secreted by a woman on the day of her delivery, together with the composition of the normal milk of the same individual.

	Colostrum.	Milk.
Water	828.0	887.6
Solid constituents	172.0	112.4
Fat	50.0	25.3
Albumen and casein	40.0	34.3
Lactose	70.0	48.2
Mineral salts	3.1	2.3

SPERMATIC FLUID.

§ 220. The spermatic fluid consists of a mixture of the secretion of the testicles and Cowper's glands, and of the mucus of the seminal vesicles. It has a characteristic odor, an alkaline reaction, and is colorless or only slightly grayish. It contains about fifteen per cent. of solid constituents, among which are a phosphorized fat, an albuminoid body, and certain mineral salts, notably the phosphates. The organized constituent of the spermatic secretion is peculiar, and characteristic, as will presently be described.

The medico-legal expert is not unfrequently called upon to decide whether certain spots upon clothing or articles of furniture have been produced by the spermatic fluid. In such a case, as Dragendorff justly remarks, the chemical properties of the spermatic secretion are of little value, for the fluid may be mixed with vaginal mucus, leucorrhœal or gonorrhœal discharges, dried urine, and other matters, which would effectually disguise the chemical reactions of the seminal secretion, even were these latter much more characteristic than they actually are.

Spots of dried spermatic fluid are evenly spread out, and usually have irregular, broken contours; their color is grayish or yellowish, and they sometimes present a

shining appearance. When seen by transmitted light they appear translucent, and, unless the fabric be very thick, the spot penetrates the tissue and is visible on each side. Linen is stiffened by the dried secretion as if by starch, but the stiffness disappears when the spot is moistened with water; at the same time the characteristic odor of the seminal secretion is developed, and may be made more marked by boiling some water in a small test-tube and causing the vapor to pass through the spot.

All of these characters are somewhat uncertain, and the only absolute proof of the presence of seminal fluid, must be derived from the detection of one or more spermatozooids, by the aid of the microscope.

The spermatic fluid is characterized by the presence of organized elements, known as spermatozooids, which may always be detected in the healthy secretion. A magnifying power of from 300 to 500 diameters is necessary for the definition of these small bodies; they are then seen to consist of an enlarged portion, called the head, and a long filamentary appendage, known as the tail (Fig. 69). The head has a somewhat flattened pear shape, being 0.005 mm. long, 0.002 mm. thick, and 0.003 wide. The examination of supposed spots of seminal secretion, must then be conducted with a view to the detection of one or more spermatozooids.



Fig. 69.

Spermatozooids.

A strip about one centimetre in width is cut from the stained fabric, embracing the whole spot, if the latter be small, or taken from the centre if it be large; in either case the extremities of the strip should extend beyond the borders of the spot. This strip is then suspended vertically, so that one end may dip into some distilled

water in a watch-glass. In the course of some time, varying from a few minutes to several hours, the water rises in the fabric, by capillarity, and the spot acquires all the external appearances of a fresh stain.

When the stain has become thoroughly softened, the surface of the fabric is gently scraped with a clean knife, and the matter thus detached is placed on a microscope slide, and carefully spread out, by the aid of a drop of water, if necessary. It is then covered with a thin glass cover, and is ready for microscopic examination.

The spermatozoids may be readily detected, either entire or broken, and in either case their identification is usually sufficiently easy. On the addition of a drop of acetic acid, the mucus is dissolved, and the form of the spermatozoids becomes more apparent. When broken, the fracture usually occurs close to the head, or in the middle of the tail.

In addition to the spermatozoids, which are conclusive evidence of the nature of the stain, the microscopic examination may reveal the presence of other bodies, derived either from the seminal secretion or from foreign sources. These are—

Fat corpuscles.

Granular and spherical mucous corpuscles.

Small, irregular bodies derived from the seminal vesicles.

Epithelial cells from the urethra.

Crystals of magnesium phosphate.

Filaments of the fabric from which the spot was removed, and foreign matters with which it may have come in contact, as well as starch granules, in case the fabric were starched linen; the starch granules, however, nearly always swollen and broken, sometimes unrecognizable with certainty.

EXCREMENTS.

§ 221. The solid excrements are highly complicated in composition, since, in addition to the intestinal mucus, which is constantly present, they contain a great variety of organized matter, derived from the food, from remedial agents, or from pathological action.

A microscopic examination reveals the presence of epithelial cells, and the morphological constituents of the intestinal mucus, vegetable cells, starch granules, muscular fibres and fat globules, derived from the various aliments. In diseased conditions of the intestines, portions of intestinal mucous membrane, blood corpuscles, and filaments of fibrin may also be encountered. Infusoria and crystals of ammonio-magnesium phosphate are frequently present, even in health.

The solid excrements of a healthy adult contain about 25 per cent. of solid matter, consisting principally of the debris of food which is insoluble, and insoluble salts.

Among the principles which are soluble in water, alcohol, or ether, are albuminoid bodies in small quantity, volatile fatty acids, lactic acid, products of the metamorphoses of the biliary acids, salts of the fatty acids proper, cholesterin, occasionally glucose, and taurine and biliary pigments, together with certain mineral salts, principally earthy phosphates. The brown color of the fæces is attributable principally to hydrobilirubin.

According to Hoppe-Seyler, the substance which Flint claimed to have extracted from the excrements, and to which he gave the name stercorin, was only impure cholesterin.

Pathologically, the fæces may also contain albumen, urea, hematin, and large quantities of sodium chloride. Hematin may exist normally in the excrements, being derived from the food, but it may also be due to hemorrhage into the intestinal canal.

Analysis of the excrements will in nearly all cases be merely qualitative. The matters are triturated with alcohol, and the alcoholic solution is separated by filtration;

the residue is exhausted with ether, the new residue treated with a little hydrochloric acid, and again exhausted with ether. The insoluble portion may then be extracted with water. The alcoholic solution will contain hydrobilirubin, fatty acids, either free or as alkaline salts, biliary acids, traces of cholesterin, and salts. The first ethereal extract will contain cholesterin and the fats which were undissolved by the alcohol, and the acid ethereal solution will contain calcium stearate and palmitate. If it be desired to estimate the proportion of mineral salts present, a known weight of the matter should be incinerated, and the ash treated as in the usual course of mineral analysis.

Albumen, which is present in the fæces in diarrhœa, may be detected by filtering the matter, after addition of a little water, if necessary, and boiling the filtrate with nitric acid.

Urea is often eliminated in considerable quantity by the bowels in cases of cholera; it may be separated by acidifying the filtered liquid with acetic acid, concentrating to a small bulk on a water-bath, and treating with nitric acid, as has been described for the preparation of urea from urine.

The ingestion of iron compounds communicates a dark-green or black color to the excrements, in which the iron seems to be present in the form of sulphide.

EXAMINATION OF THE ASH OF ANIMAL SUBSTANCES.

§ 222. In undertaking a qualitative analysis of the ashes obtained by incinerating animal substances, it must be borne in mind that the number of elementary bodies present in such substances is limited, and, as a rule, the analysis will therefore be confined to the detection of potassium, sodium, magnesium, calcium, iron, silica, sulphur, chlorine, and phosphoric, carbonic, and sulphuric acids. Besides these elements, traces of manganese, copper, lead, and fluorine may sometimes be detected.

The finely powdered ash should be boiled with distilled water in a small flask or beaker, and the liquid should then be filtered. A few drops of the filtrate are evaporated upon a piece of platinum foil, and, if any residue remain, part of the ash has been dissolved by the water, and the aqueous solution must be tested as presently described. Should no residue remain, the ash is entirely insoluble in water, and the examination is continued as in § 224.

§ 223. *The ash is partly soluble in water.*—If the aqueous solution have an alkaline reaction, basic phosphates, alkaline carbonates, or analogous substances are present: if, on the contrary, the liquid be neutral, only very small quantities of alkaline carbonates or phosphates can be present.

a) A small portion of the solution is evaporated nearly to dryness, and a few drops of hydrochloric acid are added. If effervescence take place, and if the gas disengaged produce a cloud in a drop of lime-water placed on a watch-glass and held over the mixture, carbonic acid gas is present. If no effervescence take place, no carbonic acid is present.

If the mixture smell of hydrogen sulphide, and a paper moistened with solution of lead acetate and held over the mixture be blackened, an alkaline sulphide is present; such sulphide is usually produced by the reduction of

alkaline sulphates during the incineration of the animal substance.

b) To another portion of the solution, acidified with hydrochloric acid, or the same portion which has served for the preceding tests, a drop of barium chloride is added: the formation of a white precipitate, insoluble in hydrochloric acid and in nitric acid, indicates the presence of an alkaline sulphate or of calcium sulphate. The aqueous solution used in making this test should not contain too much hydrochloric acid, for barium chloride is only slightly soluble in that acid, and might be thrown down, so leading to error. In this case, however, the barium chloride would redissolve on the addition of water. The absence of a precipitate after the addition of barium chloride, is a proof of the absence of soluble sulphates.

c) Another portion of the aqueous solution is acidulated with nitric acid, and treated with a few drops of silver nitrate. A white, curdy precipitate, insoluble in nitric acid, soluble in ammonia, and blackened by exposure to light, is due to the presence of a chloride; if no precipitate form, no chloride is present.

d) A fourth portion of the solution is treated with ammonia, ammonium chloride, and magnesium sulphate. The presence of an alkaline phosphate would then occasion the formation of a white, crystalline precipitate of ammonio-magnesium phosphate, soluble in mineral acids and acetic acid. In dilute solutions, this precipitate only separates after standing, or when the liquid is stirred.

e) A small quantity of the liquid is evaporated nearly to dryness, and the residue is tested for sodium by the flame test; sodium salts impart a bright-yellow color to the colorless flame of alcohol or a Bunsen burner, into which they are introduced on the end of a platinum wire.

f) The same concentrated liquid which was used for the detection of sodium is treated with a drop or two of hydrochloric acid, and a few drops of a strong solution of platinic chloride are added. If a light-yellow precipitate separate when the liquid is stirred, it is due to the formation of potassio-platinic chloride, and indicates the presence of potassium. In order that this test may succeed, the aqueous solution must be quite concentrated.

g) A few drops of the aqueous solution are tested with ammonium oxalate; the formation of a white, crystalline precipitate shows the presence of calcium.

§ 224. That portion of the ash which is insoluble in water is dissolved by boiling with hydrochloric acid. If an effervescence occur on first adding the hydrochloric acid, such disengagement of gas, which is without odor and produces a cloud in lime-water, indicates the presence of an earthy carbonate, probably calcium carbonate. As a rule, hydrochloric acid dissolves all of the ash, leaving a mere trace of silica, and perhaps unburned carbon; if, however, this residue have a reddish-brown color, due to the presence of ferric oxide, it should be boiled with a little concentrated hydrochloric acid containing a few drops of nitric acid.

I. The solution is filtered, some solution of ammonium chloride is added, and the mixture is rendered strongly alkaline by ammonia, and heated to the boiling point.

a) No precipitate is formed; in this case, iron, and calcium and magnesium phosphates, are not present. Proceed to II.

b) A flocculent precipitate is formed; if this precipitate be reddish-brown, or brownish-yellow, iron is present, and the whole of the iron did not previously exist in combination with phosphoric acid. If, on the contrary, the precipitate be white or only slightly yellowish, all of the iron existed as phosphate. In this case, the precipitate is separated by filtration, and the liquid is examined according to II., while the precipitate is dissolved in the smallest possible quantity of hydrochloric acid, and the solution obtained divided into several portions and tested as follows:—

(1) One portion is treated with a solution of potassium ferrocyanide; a blue precipitate (Prussian blue) indicates the presence of iron.

(2) To another portion is added a concentrated solution of sodium acetate, and the mixture is shaken violently; a flocculent, white or yellowish precipitate indicates the presence of ferric phosphate; the absence of a precipitate shows that only traces of ferric phosphate, if any, can be present.

(3) To the clear liquid, separated by filtration from the precipitate in (2), or without filtration should no precipitate have been formed, a solution of ammonium oxalate is added; a white, crystalline precipitate, soluble in hydrochloric acid, is occasioned by the formation of calcium oxalate, the calcium having probably existed as calcium phosphate or fluoride.

(4) Another portion of the solution is treated with an excess of sodium acetate and enough ferric chloride to communicate a red color to the liquid, and the latter is boiled; by this means all of the ferric phosphate and ferric oxide are precipitated. The filtered liquid is freed from calcium by the addition of ammonium carbonate, and the new filtrate is treated with sodium phosphate; a white, crystalline precipitate, formed either immediately or after standing, indicates the presence of magnesium, which existed in the ash as magnesium phosphate.

If no ferric phosphate has been detected in (2), the presence of phosphoric acid may be detected by the addition of a drop of ferric chloride, which will occasion the formation of a yellowish precipitate.

II. The solution in which no precipitate was formed by the treatment with ammonia and ammonium chloride, or the filtrate from the precipitate if any were formed, may contain magnesium and calcium which existed in the ash as carbonate.

(1) This solution, which must contain ammonia and ammonium chloride, is treated with ammonium oxalate; a white crystalline precipitate indicates the presence of calcium, originally existing as carbonate, at least not as either phosphate or fluoride.

(2) The filtrate from the precipitated calcium oxalate or the liquid in which no precipitate has been formed, is treated with sodium phosphate; the presence of magnesium will be indicated by the formation of a white crystalline precipitate, either immediately or in the course of twenty-four hours.

Quantitative Analysis of Ashes.

ESTIMATION OF POTASSIUM AND SODIUM.

§ 225. A known weight of the ash is exhausted with dilute hydrochloric acid, and barium chloride is added to the filtered liquid as long as a precipitate continues to be formed; the precipitate is separated by filtration, and washed, the washings being added to the filtrate. The latter is then treated with ammonia and ammonium carbonate, and the new precipitate is also separated by filtration. If magnesium be present, this must be precipitated by ammonium phosphate. The clear liquid, thus freed from all metals, excepting potassium and sodium, is evaporated to dryness, and the residue is heated until all ammoniacal compounds are expelled. It is then dissolved in a little water, and should any insoluble residue remain, a new filtration will be necessary; the solution of potassium and sodium chlorides is evaporated to dryness, and the residue is calcined at a low temperature, and weighed. It is then redissolved in water, platinic chloride is added until the liquid has a dark-yellow color, and the mixture is allowed to stand twenty-four hours: the precipitate of platino-potassium chloride is then collected on a tared filter, washed with alcohol, and dried at a temperature between 100 and 110°. The dry precipitate is then weighed in the filter; 100 parts of platino-potassium chloride correspond to 30.57 parts of potassium chloride. The weight of the potassium chloride so formed being deducted from that of the mixed chlorides, the remainder will represent the quantity of sodium chloride present.

100 parts of potassium chloride contain 52.41 parts of potassium.

100 parts of sodium chloride contain 39.32 parts of sodium.

ESTIMATION OF PHOSPHATE OF IRON, CALCIUM, AND MAGNESIUM.

One weighed portion of the ash serves for the estimation of phosphate of iron and also of calcium and mag-

nesium. The filtered solution of the ash in hydrochloric acid is rendered alkaline by a slight excess of ammonia, and acetic acid is added. The earthy phosphates which were at first precipitated are thus redissolved, while the ferric phosphate remains unaffected, and may be collected on a filter, washed, dried, incinerated, and weighed.

The calcium is precipitated from the filtrate by ammonium oxalate, and the calcium oxalate thrown down is collected, washed, dried, and incinerated. When cold, the residue is moistened with ammonium carbonate, and again heated to dull redness; the calcium carbonate so formed is then weighed. 100 parts of calcium carbonate contain 40 parts of calcium.

The filtrate from the calcium oxalate is then concentrated, and treated with an excess of ammonia, and a little sodium phosphate. After standing twenty-four hours, the precipitated ammonio-magnesium phosphate is collected on a filter, washed with dilute ammonia (1 part ammonia to 3 parts water), and dried. The dry precipitate should then be introduced into the crucible, and the filter burned separately, over a piece of glazed paper. The ash is added to the matter in the crucible, and the whole is ignited, first gently, finally at a white heat. The residue, consisting of magnesium pyrophosphate, is then weighed. 100 parts of magnesium pyrophosphate contain 21.62 parts of magnesium, and correspond to 78.67 parts of normal magnesium phosphate, in which form the magnesium usually exists in the ash.

ESTIMATION OF SULPHURIC ACID.

Sulphuric acid is estimated in the form of barium sulphate. The solution of a weighed quantity of the ash is treated with an excess of barium chloride, and the mixture is heated to boiling. The precipitated barium sulphate is then collected on a filter, and washed with hot water; part of the barium sulphate is apt to pass through the filter if cold water be employed. The precipitate is then dried, calcined, and weighed. 100 parts of barium sulphate correspond to 42.06 parts of sulphuric acid, or to 41.20 parts of SO^4 .

ESTIMATION OF CHLORINE.

Chlorine may be estimated volumetrically, as described in § 154, or by the balance. In the latter case, one or two grammes of the ash are digested with about an equal quantity of pure sodium carbonate dissolved in distilled water; after standing some time, the mixture is evaporated to dryness, and the residue is exhausted with water, which dissolves the alkaline salts, while all of the earthy salts remain undissolved. The solution is acidulated with nitric acid, gently heated, and precipitated by adding silver nitrate. The silver chloride which is thrown down is collected on a filter, and washed with distilled water, until the washings show no turbidity on the addition of hydrochloric acid. The precipitate is then dried in the filter, and when quite dry is turned out on glazed paper, and the filter is burned in a porcelain crucible. After cooling, since part of the silver chloride adhering to the filter-paper will have been reduced, a few drops of nitric acid and about as much hydrochloric acid are added, and the mass is evaporated to dryness on a water-bath. The precipitate which was left on the glazed paper is then added, and the whole is cautiously heated until it fuses. After cooling, the fused silver chloride is weighed, 100 parts of it corresponding to 24.74 parts of chlorine.

ESTIMATION OF PHOSPHORIC ACID.

A weighed quantity of the ash is dissolved in hydrochloric acid, and the filtered solution is treated with ammonia, ammonium chloride, and magnesium sulphate. After standing twenty-four hours, the precipitated ammonio-magnesium phosphate is collected, washed, dried, and incinerated, observing the precautions necessary in estimating magnesium. 100 parts of magnesium pyrophosphate correspond to 63.39 parts of phosphoric anhydride, P^2O^5 .

Should it be necessary to estimate carbonic acid, the operations are conducted as described in § 200, *b*.

PART III.

ON THE DETECTION OF POISONS.

§ 226. In cases of suspected poisoning not followed by death, the matters subjected to chemical analysis will be limited to the residue, if any, of the suspected substance, and to the urine and matters vomited by the patient. Should the poisoning terminate in death, the analysis should also include the stomach and its contents, the intestines and their contents, the liver, bile, spleen, pancreas, blood, and urine if the bladder be distended. In certain cases it may also be advisable to examine the brain, kidneys, and even the lungs.

In any case, the materials should be immediately placed in clean glass jars, and labelled and sealed, until the chemical examination is to be undertaken. No disinfectant or preservative of any kind should be employed, as such addition would preclude a research for the body used, and might prevent the detection of certain poisons.

Each organ or substance to be examined should be analyzed separately, for all poisons are not equally absorbed by the system, and, after absorption, all are not eliminated through the same channels; certain substances may be detected in the urine within a very short time after their ingestion, and the examination of that liquid may be of great importance and should not be neglected; this is especially the case in poisoning by antimony.

Each organ should be finely divided separately, and the mass rendered as homogeneous as possible by thorough mixing. Since accidents are not impossible even in the most experienced and most careful hands, and since questions of accuracy may arise, only half of the suspected material should be submitted to chemical ex-

amination, the other half being resealed and reserved for unforeseen emergencies.

It is of the utmost importance that the chemical reagents and apparatus used in examinations for poisons should be absolutely pure and clean. All of the reagents employed must be tested in the same manner as in the examination for the poison, and must be changed or properly purified should any trace of impurity be found.

§ 227. As a rule, the chemist has received some indications on the probable nature of the suspected poison before undertaking a toxicological investigation, these indications being furnished by the history of the case, certain post-mortem appearances if death have followed the poisoning, and the smell, taste, color, or other physical property of the substance under examination. In such a case, the labor of the analyst is often comparatively light; but in some cases no such aids are presented, and it becomes necessary that a complete and systematic investigation shall be made, covering all or as many as possible of the poisonous substances which are known. The examination must then be so conducted that no part of the substance is wasted, and, for this purpose, volatile and easily destructible poisons, together with those of organic origin, are first sought, and the residues from this examination are examined for the presence of mineral poisons, which would be unaffected by the previous operations.

We will first consider the detection of the more common poisons individually, supposing that circumstances have pointed to one or the other of these as probably present; we will then summarily describe the method to be followed when the chemist has no idea of the particular poison present, and when a preliminary examination has given no affirmative result.

ARSENIC.

§ 228. Among the metals which it may be necessary to seek in chemico-legal examinations, arsenic is the most prominent. The well-known poisonous properties of its compounds, their want of smell, taste, and in many cases color, and their easy accessibility to the public, have combined to render this element one of the most frequent instruments of suicide and of criminal and accidental poisoning.

§ 229. The more usual forms in which arsenic is met with are as follows:—

METALLIC ARSENIC, in the impure state often called cobalt, and used as a fly poison. This is insoluble in water, but slowly oxidizes in the air, and when kept under water becomes partially oxidized by the air dissolved in the water, so that the commercial article always contains more or less arsenious oxide.

ARSENIOUS OXIDE, commonly known as white arsenic or rats' bane.

ARSENIC DISULPHIDE, or realgar, a red solid, and

ARSENIC TRISULPHIDE, or orpiment, a yellow solid, used as pigments.

GREEN PIGMENTS containing various compounds of arsenic and copper, among which are *Scheele's green* (copper arsenite), and *Schweinfurth's green* (a compound of arsenite and acetate of copper).

POTASSIUM ARSENITE, used in medicine under the name *Fowler's solution*.

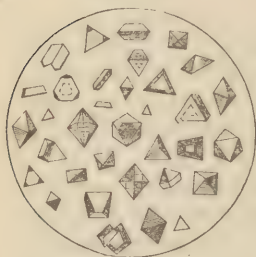
Although all of these substances, as indeed nearly all of the compounds of arsenic, are more or less poisonous, our attention will be particularly directed to arsenious oxide, since it is in this form that the poison is generally encountered in toxicological analysis. Again, the processes which serve for the detection of arsenious oxide are, when slightly modified, equally applicable to the detection of the other compounds.

§ 230. The presence of arsenic trisulphide or of either of the arsenical pigments, is usually at once indicated by

particles of colored powder, which sometimes tinge the entire mass. If yellow particles be found, they are carefully collected together, thoroughly dried, and mixed with a little powdered potassium cyanide; the mixture, which must be quite dry, is introduced into a small tube of hard glass, closed at one end, and is heated to redness. If arsenic be present, a brilliant metallic mirror will be formed in the cooler part of the tube; this is then examined as directed farther on (§ 233, *a*). Should no colored particles be found, but the presence of one of the arsenic pigments be still suspected, the substance is treated according to section 236.

IDENTIFICATION OF ARSENIUS OXIDE UNMIXED WITH OTHER SUBSTANCES.

§ 231. Arsenious oxide commonly occurs as a white powder or in lumps. It is not very soluble in water; one part of the opaque, crystalline compound, as it usually occurs in commerce, requires about seventy-five parts of water for its solution. Hence, when solid arsenious oxide has been administered, white grains of the undissolved substance are frequently found in the contents of the stomach or in vomited matter. The examination is then easy, and the first results should be decisive.



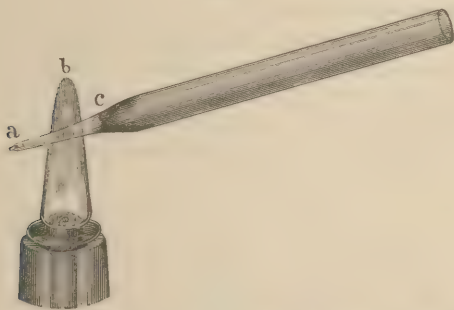
Crystals of arsenious oxide.

§ 232. Some of the powder is cautiously heated in a small tube of hard glass, closed at one end, slightly warming the upper part of the tube before heating the suspected substance. Arsenious oxide will in this case sublime, forming a white or colorless crystalline sublimate, which, when examined by the aid of a microscope or good lens, will be seen to consist of brilliant octahedral crystals, and forms derived from an octahedra (Fig. 70). In order that the form of the crystals may be well marked, the tube must be heated

slowly; if this be neglected, it may be impossible to obtain any regular crystals.

§ 233. A small quantity of arsenious oxide mixed with dry sodium carbonate and powdered charcoal, and heated in a small tube closed at one end, will be reduced, and a brilliant mirror of metallic arsenic will be formed in the cooler part of the tube. This experiment, known as the reduction test, is best performed as follows: a small tube of hard glass is drawn out to a long point, which is then sealed. The suspected powder is introduced into the point of the tube, and a small splinter of dry charcoal is placed in the drawn-out portion, about one centimetre above it. The part of the tube containing the charcoal is now heated to redness, and the tube is shifted so as to bring the end containing the powder into the flame. The arsenious oxide instantly volatilizes, and, passing over the incandescent charcoal, is reduced to metallic arsenic which condenses in a brilliant mirror farther on in the tube (Fig. 71).

Fig. 71.



a) The drawn-out end may then be cut off at *a*, the charcoal removed, and the tube again sealed. By carefully moving the tube back and forwards in the flame, the arsenic may be again volatilized, and oxidized by the air in the tube into arsenious oxide, which condenses in octahedral crystals in the cooler part of the tube. If preferred, that part of the tube containing the mirror

may be crushed, and the fragments heated in another tube.

§ 234. The following tests, known as the liquid tests, are applied to solutions suspected to contain arsenious oxide. A portion of the white powder is dissolved, by the aid of heat, in distilled water, and the tests are applied to separate portions of the solution so obtained.

a) Hydrogen sulphide passed through a solution of arsenious acid acidulated with a drop or two of hydrochloric acid, occasions the formation of a bright yellow precipitate of arsenic trisulphide. This precipitate is readily soluble in ammonia. It may be further examined by § 230.

b) Ammonio nitrate of silver causes in solutions of arsenious acid, a canary-yellow precipitate of silver arsenite, soluble in nitric acid and in ammonia. The test is best made by first adding solution of silver nitrate, then a drop of dilute ammonia to the suspected liquid. In the presence of a chloride, this test is without value.

c) When ammonio-sulphate of copper is added to a solution of arsenious acid, a pale green precipitate of copper arsenite is formed.

In the course of a toxicological investigation, the crystals of arsenious oxide obtained by one of the preceding methods, or by one of those yet to be described, may be dissolved, by crushing the tube containing them and boiling the fragments with water, and the liquid tests applied to the solution so obtained.

THE ARSENIC EXISTS IN AN ORGANIC MIXTURE.

§ 235. If the matters suspected to contain arsenic be liquid, they may be acidulated with hydrochloric acid, and at once submitted to Reinsch's test or to Marsh's test (see farther on). If they consist of both solid and liquid, the latter should be separated as much as possible from the former, and the two portions examined separately. The liquid, if not too viscid, may be tested by Reinsch's or Marsh's test, and, if negative results be obtained after long and repeated trials, it may be assumed that no arsenic is present. If the presence of arsenic be

indicated by these preliminary tests, the organic matter should be destroyed, and the examination continued as will presently be described. Solid or semi-solid substances, such as the contents of the stomach or vomited matters, should be examined for small particles of arsenious oxide, which, if found, should be picked out, and identified as in §§ 231-234.

The solid or semi-solid matter is then digested at a moderate temperature, on a water-bath, with dilute hydrochloric acid (containing one-eighth or one-tenth acid); the solution obtained after filtration may be examined by Reinsch's, Marsh's, or Bloxam's test. The solid residue is treated as directed in § 236.

In medico-legal investigations as to the presence of arsenic, it is absolutely necessary, in case none of the poison can be detected in the stomach and its contents, to examine the various tissues of the body; since the poison, when introduced into the stomach during life, is absorbed and gradually diffused throughout the whole system, and may be found in the blood, urine, muscles, and viscera, especially in the liver. It is therefore advisable to examine each of these for the poison, and it should never be concluded that because it cannot be detected in the stomach and its contents, none is to be found in other parts of the body. Should the individual, however, survive during several days after swallowing the poison, it is possible that the whole of it may be eliminated from the body, in which case, of course, no trace of it could afterwards be detected.

If the solid matter to be examined have undergone putrefaction, the arsenious oxide may have become partially converted into arsenic trisulphide, which is sometimes perceived in bright-yellow patches (see § 230).

§ 236. The solid matter intended for examination is cut up as finely as possible with a knife or scissors, and heated in a porcelain or glass dish, on a water-bath, with about an equal weight of pure hydrochloric acid; powdered potassium chlorate is added from time to time, and the mixture stirred until the solid matter is entirely broken up, and the mass is fluid enough for filtration. Should the hydrochloric acid added be insufficient to

render the mass fluid, distilled water may be added until a proper consistence is attained. After thus destroying the organic matter by hydrochloric acid and potassium chlorate, an almost clear solution is often obtained,—cellular tissue, fat, and ligneous fibres, alone resisting the action of the chlorine evolved. (Fresenius and Babo.)

The excess of chlorine is expelled by evaporation on a water bath, or by passing a current of carbonic acid gas through the liquid. The bath is then filtered hot, distilled water being added before filtration, should the original volume of the liquid have been much reduced; the matter on the filter is washed with distilled water, and the washings are added to the filtrate.

The filtrate, which should not smell of chlorine, is introduced into a flask, and washed sulphurous acid gas is passed through it, until it smells strongly of the gas. Arsenic acid, into which any arsenic compound originally present would have been converted by the treatment with potassium chlorate, is thus reduced to arsenious acid. The solution is again heated on a water-bath until all of the sulphurous gas is expelled, and a portion of it may then be submitted to Marsh's, Reinsch's, or Bloxam's test.

§ 237. Hydrogen sulphide is passed through the liquid until the latter possesses a strong odor of the gas, even after standing some time, and the precipitated arsenic trisulphide is collected on a filter, and thoroughly washed with distilled water. It is then dried, and, with the filter, is spread out in a porcelain capsule, sprinkled with concentrated nitric acid, and evaporated to dryness; the residue is again moistened with nitric acid and evaporated, and these operations are repeated until the residue acquires a yellowish color. It is then dissolved in a very little sodium hydrate solution, and well mixed with finely powdered sodium carbonate and a little sodium nitrate. The mixture is introduced into a porcelain crucible, and the capsule rinsed out with a little dry sodium carbonate, which is added to the matter in the crucible; the contents of the latter are thoroughly dried, and gradually heated over a burner. The mass blackens, then becomes decolorized without deflagration, and melts

to a colorless liquid. In case this liquid be not colorless, a little more sodium nitrate must be added.

§ 238. The fused mass will contain sodium arsenate, together with nitrate, nitrite, sulphate, and carbonate of sodium. When cold, it is treated with the smallest possible quantity of warm water, and a little sodium acid carbonate is added. Any antimony present would remain undissolved as sodium antimonate, while tin would be precipitated as oxide by the sodium acid carbonate. The soluble portion, which contains sodium arsenate, should the original substance have contained arsenic, is separated from the insoluble residue, and the latter washed with distilled water, the washings being added to the filtrate. The residue may be used for the detection of antimony and tin, if the presence of those metals be suspected. The filtrate is strongly acidulated with dilute sulphuric acid, care being taken that no loss occur by effervescence, and the liquid is evaporated in a porcelain capsule until all of the nitrous compounds are expelled, as is indicated by the appearance of heavy vapors of sulphuric acid.

The liquid remaining in the capsule is directly used for Marsh's or Bloxam's test; before the application of Reinsch's test it should be boiled with some sulphurous acid solution; but, as the metal is obtained directly by Marsh's test, positive results yielded by that method, and confirmed by the crystalline sublimate and liquid tests, are sufficient evidence of the presence of arsenic.

§ 239. *Detection of cupro-arsenical pigments in paper hangings and other fabrics.*—The cupro-arsenical pigments may be dissolved by soaking the suspected material in aqueous ammonia; if copper be present, the solution will have a blue color, and arsenic may be detected by acidulating with hydrochloric acid, and boiling with copper foil, as directed in the following section.

Reinsch's Test.

§ 240. This test depends upon the fact that a solution of arsenious acid in hydrochloric acid deposits a gray coating on a copper slip or wire immersed in it; this coating is formed more or less quickly, according to the amount of arsenic present, and the temperature of the solution. Lippert has shown that it does not consist of pure arsenic, but is a compound containing five atoms of copper and two atoms of arsenic, As_2Cu_5 .

In the application of this test, which is one of the most delicate known for arsenic, the hydrochloric acid and copper used must be of absolute purity. To insure this, some of the hydrochloric acid is diluted with about four times its volume of water, and boiled for fifteen or twenty minutes with the slip of copper to be used. Should the copper then remain clean and bright, it may be assumed that both are pure. However, several small strips of the copper may be strongly heated in a small tube of hard glass in order to see that no crystals of arsenious oxide are deposited on the sides of the tube.

The liquid to be tested should contain no sulphurous acid, and as the latter is frequently employed to reduce arsenic compounds to the arsenious condition, care must be taken that all of the gas is expelled from the liquid by boiling before applying Reinsch's test.

Although it is generally advisable to destroy all organic matter before proceeding to the detection of a mineral poison, good results may be obtained by directly submitting to Reinsch's test the liquid produced by digesting the suspected matters with hydrochloric acid diluted with about five times its volume of water.

In order to apply this test, acidify a portion of the solution suspected to contain arsenic with a little hydrochloric acid; heat to boiling, and, as soon as the liquid begins to boil, introduce one or more small strips of bright copper foil that has been found free from arsenic. If arsenic be present, it will form a steel-colored coating on the copper, and may be identified in the following manner:—

a) Carefully wash the copper strips, boil them with a little alcohol should any fatty matter have been present in the suspected liquid, and dry them by pressure between folds of filter-paper, or by a gentle heat. Then introduce them into a small tube of hard glass, closed at one end, and heat, first gently, then strongly, so that the arsenic may volatilize, and, coming in contact with the air in the tube, may be oxidized to arsenious oxide, which will condense in octahedral crystals on the sides of the tube. The crystals are identified under the microscope.

b) Remove the copper from the tube, and crush that portion of the latter containing the sublimate, taking care that the crystals be not detached. Boil the fragments with as small a quantity of distilled water as possible, and examine the solution so obtained by the liquid tests (§ 234 *a* and *b*). Or, unless the tube be very narrow, the closed end may be cut off, and the whole tube boiled with water in a test-tube, the test being applied as before.

The difference between the deposit obtained from arsenic by Reinsch's test and that produced by antimony, will be considered when treating of the latter metal.

Should arsenic be present in the form of arsenic acid, this must be reduced by sulphurous acid, and the excess of the latter entirely expelled, before applying Reinsch's test.

Marsh's Test.

§ 241. When a solution of arsenious oxide, an arsenite, or an arsenate, is submitted to the action of nascent hydrogen, the arsenic compound is reduced, and the arsenic combines with the hydrogen, forming hydrogen arsenide, a poisonous gas which must be handled with care.

This reaction is applied to the detection of arsenic by Marsh's apparatus, as it has been modified by Berzelius. Various sources of nascent hydrogen have been proposed; potassium hydrate and aluminium,¹ sodium amalgam and water,² have been employed by different chemists, but the method originally employed by Marsh, the action of

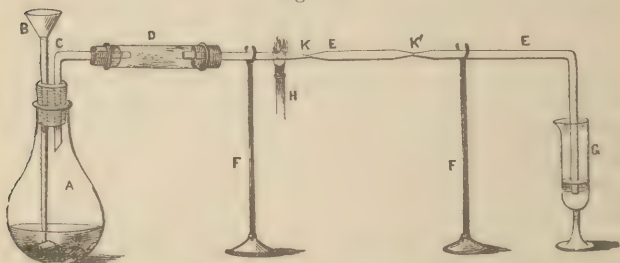
¹ Gatehouse, 1873.

² Naquet, 1873.

zinc upon dilute sulphuric acid, is by far the most convenient for practical purposes, and, when proper care is taken to insure the purity of the reagents, is open to no well-founded objection.

A gas bottle (Fig. 72) is arranged for the preparation of hydrogen, the delivery-tube KE being of hard glass,

Fig. 72.



and so connected with the bent tube D that the jet may be turned either up or down. The wider tube D may contain some cotton-wool destined to dry the gas.

Some pure zinc is placed in the bottle, and when all is in readiness cold dilute sulphuric acid, containing about one-sixth acid, is introduced through the funnel-tube. After sufficient time has been allowed for the expulsion of the air from the bottle, the gas is lighted at the jet, and a piece of clean porcelain depressed into the flame. If, after repeated trials, no spots or stains are formed on the porcelain, it may be assumed that both zinc and acid are pure, and some of the suspected liquid is introduced by the funnel-tube. The gas should not be extinguished while pouring in the liquid, but care should be taken that no bubbles of air enter. If frothing occur after the introduction of the suspected liquid, it may be checked by pouring in a little alcohol. Should any quantity of arsenic be present, the appearance of the flame undergoes a striking change; from almost colorless, it becomes pale-blue, and emits fumes of arsenious oxide. Whether the appearance of the flame be changed or not, a piece of clean porcelain (a crucible lid or piece of a broken capsule answers well) is depressed into the

flame, and, should the liquid have contained arsenic, dark spots will be deposited on the porcelain. As many of these deposits as possible are obtained, and are subjected to the following tests in order to ascertain whether they be really arsenic or be due to antimony, which would produce very similar results.

a) The arsenic spots will volatilize when gently heated over a lamp, and garlicky odors of arsenic will be perceptible. Antimony spots are not easily volatilized.

b) Arsenic spots are but slowly dissolved by yellow ammonium sulphhydrate; antimony spots are dissolved immediately.

c) Arsenic spots are at once dissolved by solutions of the hypochlorites (sodium hypochlorite, chlorinated lime); antimony spots are affected very slowly by the same reagents.

d) If the spot be moistened with a drop of strong nitric acid, and then carefully heated until the excess of acid is expelled, a white residue will be obtained should the spot consist of either arsenic or antimony. This is touched with a drop of silver nitrate solution, and a drop of ammonia on the end of a glass rod is held near the spot: if the latter be arsenic, a brownish-red color is produced, either before or after the application of the ammonia, due to the formation of silver arsenate. Antimony spots give no color when treated in this manner, but in order that the reaction may succeed with arsenic, care is necessary in applying the reagents.

e) While the gas is being disengaged, and burning at the jet, heat the hard glass delivery-tube at the point K. If arsenic be present, a metallic mirror or ring will be formed a little farther on in the tube, and, should this ring consist of arsenic, it may be readily driven up and down the tube by moving the lamp. At the conclusion of the experiment the portion of the tube containing the mirror is cut off, broken up, and carefully heated in a small tube closed at one end: after identifying the sublimate of arsenious oxide so obtained, it is examined as directed in section 240 *b*.

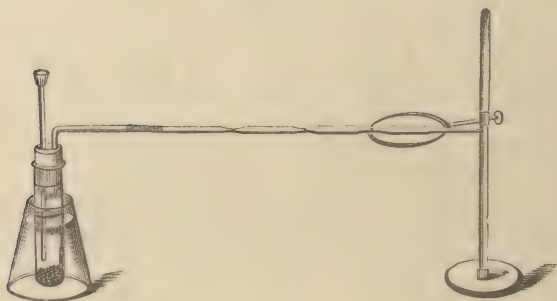
The gas is extinguished at the jet, the latter is turned down, and the escaping gas made to pass through a solu-

tion of silver nitrate. Should arsenic be present, the silver nitrate is reduced, and metallic silver is deposited as a brownish or black precipitate, while arsenious acid is formed and remains in solution, together with the excess of silver nitrate if an excess have been employed. The solution is filtered from the precipitated silver, a few drops of silver nitrate are added to the clear filtrate, and very dilute ammonia is added, drop by drop, by the aid of a glass rod. A canary-yellow precipitate of silver arsenite at once indicates the presence of arsenious acid.

Under these circumstances, hydrogen antimonide also causes the formation of a brown or black precipitate in solutions of silver nitrate, but this precipitate consists of a compound of antimony and silver, and the addition of silver nitrate and ammonia after filtration, yields no results.

When only a small quantity of a substance is to be tested for arsenic, the apparatus represented in Figure 73, and in which the flask is replaced by a tolerably large

Fig. 73.



test-tube, may be employed. The delivery-tube is contracted at several points, and its end drawn out so as to form a very narrow tube about five centimetres long. The wider parts of the contracted portions are heated by a spirit lamp or Bunsen burner, and, in order to insure the recovery of all of the arsenic, the heat is applied before the suspected substance is introduced. As many rings or mirrors are obtained as possible, and these are

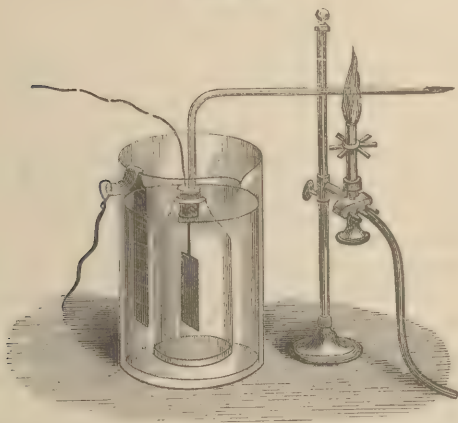
tested as in § 233, *a*. When the quantity of substance for analysis is very small, no attempt should be made to obtain spots on porcelain, as some of the arsenic is then always volatilized and lost.

The flask or test-tube may be immersed in cold water, and if the disengagement of gas be very slow, drying may be unnecessary.

Electrolytic Test.

§ 242. This exceedingly delicate test has been improved and modified by Bloxam, by whose name it is now generally known. It depends upon the fact that when a tolerably strong electric current is made to traverse a

Fig. 74.



solution containing arsenic, hydrogen arsenide is evolved at the negative pole, together with the hydrogen of the decomposed water.

The apparatus devised by Bloxam consists of a tubulated bell-jar of 50 or 100 cubic centimetres capacity, the bottom of which is closed by a piece of parchment paper tightly bound over the edges. To the tubulure of the bell-jar is adapted a cork pierced with two holes, through one of which passes a funnel-tube which

nearly touches the paper bottom, while the other gives passage to a delivery-tube bent at a right angle (Fig. 74). A strip of platinum foil cut in the shape of a spade is secured between the cork and the neck of the jar, so that its broad end, which is in the bottle, almost touches the diaphragm, while the other end projects several centimetres beyond the tubulure. The small bell-jar is then placed in a cylindrical jar, not much wider than itself, and not so flat at the bottom as to close the bell-jar. Between the exterior and interior vessels is suspended a second strip of platinum, similar to the first, and nearly touching the bottom of the jar. By means of a caoutchouc joint, the bent delivery-tube is connected with a narrow tube of infusible glass, drawn out to a fine point about five centimetres long. This tube must be properly supported, so that it may be heated at the point at which its diameter begins to contract.

The apparatus is charged with sulphuric acid diluted with three times its weight of water, both the external and internal vessels being about one-quarter filled. The whole is then placed in a basin of water to prevent too great a rise in temperature, and the platinum strip in the bell-jar is connected with the zinc or negative pole of a voltaic battery, while that suspended in the cylindrical jar is connected with the positive pole. Ten or fifteen minutes having been allowed for the expulsion of the air in the bottle by the hydrogen evolved, the shoulder of the drawn-out tube is heated to dull redness, and maintained at that temperature for ten or fifteen minutes; should no ring of arsenic or of arsenic trisulphide be then visible in the narrow part of the tube, the sulphuric acid is pure, and the solution to be tested for arsenic is slowly poured down the funnel-tube, care being taken to avoid the introduction of air bubbles. Should frothing occur, it may be checked by the introduction of a few cubic centimetres of alcohol. If no deposit of arsenic or its sulphide be formed within ten minutes, 2 c. c. of a solution of sulphurous acid or hydrogen sulphide are poured in by the funnel-tube, and the experiment is continued for another quarter or half hour. Should arsenic be present, a metallic ring of arsenic, or a greenish-

yellow, iridescent ring of arsenic trisulphide, will be deposited in the narrow part of the tube. Should no odor of hydrogen sulphide be perceptible at the end of the tube at the termination of the experiment, a little more sulphurous acid or hydrogen sulphide should be added.

The lamp is now removed, and the tube allowed to cool, after which the part containing the deposit is cut off, and gently warmed, in a small test-tube, with a solution of ammonium carbonate, which slowly dissolves the yellow sulphide. The tube with the metallic portion of the deposit is washed with distilled water, dried, and treated according to section 241 *e*.

The whole of the arsenic is seldom removed from the liquid by electrolysis. The remainder may be extracted by saturating with hydrogen sulphide, and gently heating in a covered vessel for several hours. The precipitated arsenic trisulphide, mixed with organic matter, is collected on a filter, washed, and treated as directed in section 237.

Quantitative Estimation of Arsenic.

§ 243. It is often a matter of importance to determine the absolute quantity of arsenic present in a mixture or in an organ, such as the liver. The operation is conducted precisely as indicated in §§ 236–238, except that the solution of sodium arsenate is not treated with sulphuric acid, but with ammonium chloride, ammonia, and magnesium sulphate, and the mixture is allowed to stand twenty-four hours. The arsenic will then be completely precipitated as ammonio-magnesium arsenate; this is collected on a tared filter, washed with dilute ammonia, dried at 100° , and weighed. Ammonio-magnesium arsenate contains $\text{MgNH}_4\text{AsO}_4 + \frac{1}{2}\text{H}_2\text{O}$; consequently 100 parts of the precipitate represent 39.47 parts of arsenic, or 52.1 parts of arsenious oxide.

ANTIMONY.

§ 244. The only form in which antimony is commonly met with, is the double tartrate of antimony and potassium, generally known as tartar-emetic or tartarized antimony. It enters into a number of medicinal preparations, and has not unfrequently been used as a poison. It contains $K(SbO)C^4H^4O^6$.

In the crystalline state, tartar-emetic occurs in rhombic octahedra, which contain one molecule of water of crystallization, and effloresce in dry air. It is soluble in about fifteen parts of cold water, and in about two parts of boiling water, insoluble in alcohol.

When in the pure state, tartar emetic may be recognized by the following properties: —

a) Hydrogen sulphide produces an orange-colored precipitate of antimony trisulphide. This precipitate is insoluble in ammonia, but dissolves in ammonium sulphide.

b) With hydrochloric acid, solutions of tartar emetic give a white precipitate of antimony trioxide, soluble in an excess of acid, but reprecipitated on the addition of water.

c) When a few drops of a solution of antimony acidulated with hydrochloric acid are poured upon platinum foil, and a piece of zinc is placed in the liquid, a black deposit of antimony is formed on the foil. This deposit is insoluble in hydrochloric acid; if it be moistened with yellow ammonium sulphide, and the solution be evaporated to dryness, an orange-colored residue of antimony sulphide will remain. This should be compared with the residue left upon the foil by ammonium sulphide alone.

§ 245. In the presence of organic matter, antimony is detected by processes similar to those followed in the case of arsenic. The organic matter is destroyed in the same manner, and the solution obtained may be directly submitted to Marsh's or to Reinsch's test. The

differences of the results obtained from those yielded by arsenic are as follows:—

a) When an antimonial solution is boiled with copper and hydrochloric acid, a bluish-black or purple deposit of antimony is formed on the copper. When the latter is washed, dried, and heated in a tube, it rarely gives a crystalline sublimate which can be mistaken for arsenious oxide; but on strongly heating, a white sublimate is formed, and though this is generally amorphous, it must be borne in mind that arsenious oxide and antimonious oxide are isodimorphous, and it occasionally happens that the antimonial sublimate presents, under the microscope, such a crystalline appearance as might lead to an inference of the presence of arsenic. However, this deposit is insoluble in water, and when it is boiled with water in a test-tube and ammonio-nitrate of silver is added to the liquid, no canary-yellow precipitate is formed.

When the copper coated with the antimonial deposit is boiled with a very dilute solution of potassium hydrate, slightly colored with potassium permanganate solution, potassium antimonate is formed; the solution may then be filtered from deposited manganese oxide, and treated with hydrogen sulphide and hydrochloric acid. An orange-colored precipitate of antimony sulphide will be formed. This test is quite delicate, and is trustworthy, thus furnishing a ready method for the identification of antimony.

b) With Marsh's test, antimony yields stains on porcelain, and metallic rings analogous to those produced by arsenic, from which, however, they may be readily distinguished.

1. The antimony spots and rings are much less volatile than those of arsenic, and the rings are deposited nearer the heated portion of the tube. They are not readily driven up and down by moving the source of heat.

2. Antimony spots are not easily dissolved by solutions of sodium hypochlorite or chlorinated lime. Arsenic spots are at once dissolved by these reagents.

3. Antimony spots dissolve readily in yellow ammonium sulphide, and the solution, when evaporated, leaves

an orange-colored residue of antimony sulphide ; arsenic spots are not easily dissolved by ammonium sulphide.

4. Antimony spots, when oxidized by nitric acid and evaporated to dryness, leave a residue which does not produce a brownish-red color with ammonio-nitrate of silver, as do spots of arsenic.

In Bloxam's test by the electrolytic method, hydrogen antimonide is evolved much less readily than hydrogen arsenide, the greater part of the antimony being deposited as a black coating on the platinum plate connected with the negative pole of the battery. If the plate be washed, and gently heated with a little yellow ammonium sulphide, the antimony dissolves, and an orange-colored residue of antimony sulphide is left after the evaporation of the solution.

Separation of Antimony from Arsenic.

§ 246. Should both antimony and arsenic be present in an organic mixture, the precipitate formed by passing hydrogen sulphide through the liquid obtained after the destruction of organic matter, as indicated in § 236, will contain both metals in the form of sulphides. If this precipitate be thoroughly washed with distilled water, until all traces of hydrogen sulphide are removed, the arsenic sulphide may be dissolved by pouring ammonia on the precipitate in the filter, and will pass through, while the insoluble antimony sulphide will remain. The latter may then be washed, dissolved by boiling with hydrochloric acid, and examined by Marsh's test if desired. If much sulphur be present with the precipitated sulphides, or if the hydrogen sulphide be not entirely removed by washing, part of the antimony sulphide will be dissolved by the ammonia, and the separation will consequently be more or less imperfect.

If arsenic and antimony be present together, the whole of the latter will remain as insoluble sodium antimonate when the mixed sulphides are heated with a mixture of sodium carbonate and sodium nitrate, as directed in § 238, while the sodium arsenate formed at the same time is dissolved when the fused mass is treated with water. The

insoluble antimonie salt is collected on a filter, washed, dried, and fused with five or six times its weight of potassium cyanide in a small porcelain crucible. Metallic antimony is then formed, and collects in a button at the bottom of the crucible. The cold mass is, therefore, treated with water, and the button removed: if a quantitative estimation is desired, this button is weighed before proceeding to its identification by chemical tests. 100 parts of antimony correspond to 266 parts of dry tartar-emetic.

TIN.

§ 247. Although tin and its compounds are neither common nor dangerous poisons, for several reasons the analyst must be familiar with their more important reactions. Stannous chloride, SnCl_2 , is largely used in dyeing, and has been employed criminally as a poison; and certain reactions of arsenic and antimony resemble those of tin to such an extent that it is of importance to be able to distinguish between these metals.

Tin is separated from organic mixtures exactly as the two metals previously considered. After the destruction of organic matter by potassium chlorate and hydrochloric acid, tin will remain in the liquid in the form of stannic chloride. When hydrogen sulphide is passed through the solution, pale-yellow stannic sulphide is precipitated, and might easily be mistaken for arsenic trisulphide; but, like antimony sulphide, this precipitate is insoluble in ammonia, and may be separated from any arsenic that might be present at the same time, by thorough washing with distilled water and subsequent treatment with ammonia.

The precipitate of stannic sulphide is but partially reduced by potassium cyanide, and if the mixture be heated in a glass tube, no metallic ring or mirror is formed.

When stannic sulphide is fused with potassium nitrate, it is converted into soluble potassium stannate, but with

sodium nitrate it yields sodium stannate, which is insoluble, especially in hot water. When these stannates are dissolved in sulphuric acid, and introduced into Marsh's apparatus, no spots or rings are obtained, but metallic tin remains in the gas bottle after the zinc is all dissolved. As tin is not entirely insoluble in dilute sulphuric acid, the residue in the gas-bottle should be filtered before all of the zinc has disappeared; the particles of tin are then collected, and any that adhere to the zinc are removed by washing. They are then dissolved in a little warm hydrochloric acid, and the presence of tin may be detected in the solution.

In addition to the hydrogen sulphide test before mentioned, if some of the solution of stannous chloride be poured into a solution of mercuric chloride, a white precipitate of mercurous chloride is formed, which changes to gray, if enough tin be present, owing to the separation of finely divided metallic mercury.

MERCURY.

§ 248. After arsenic, mercury is the mineral poison most frequently met with in toxicological investigations. The compounds of which it forms part, and in which it may be either accidentally or criminally administered with poisonous effects, are numerous; metallic mercury and its amalgams are employed in the arts; its chlorides and oxides are used in medicine, as are also its iodides and other salts of less importance. Of all these compounds, mercuric chloride, or, as it is commonly called, corrosive sublimate, is that by which life has been most often destroyed or endangered.

When pure, mercuric chloride is a white, crystalline solid, which is soluble in about nineteen parts of cold water, and also soluble in alcohol and ether. It is deposited from its hot, saturated, aqueous solution, in right-rhombic, anhydrous prisms. Its solutions respond to the tests indicated further on.

SEPARATION FROM ORGANIC MIXTURES.

§ 249. When the presence of mercury is suspected in organic mixtures, such as vomited matter, or the contents of the stomach, if the solid and liquid portions of the matter can be readily separated from each other, a decisive preliminary test may be made on the liquid; or the whole mass may be at once treated as indicated in section 254.

§ 250. If the liquid is to be treated separately, it is acidulated with hydrochloric acid, and boiled for quarter or half an hour with one or more pieces of clean, bright copper foil or wire. If mercury be present in the solution, it will be separated, and deposited as a gray film on the surface of the copper. The latter is then removed from the liquid, washed with a little alcohol and dilute ammonia to remove fatty matters and any adhering acid or salt of copper, and carefully dried by pressure between folds of filter-paper; it is then distributed in several small, hard-glass tubes, closed at one end. The end of the tube containing some of this coated copper being heated in a lamp-flame, the mercury volatilizes and condenses in the cooler part of the tube, forming a grayish ring in which globules of mercury may often be seen by the naked eye,—nearly always by the aid of a good lens.

§ 251. If, however, only a trace of mercury be present, the globules may not be distinguishable. In this case the copper is shaken out of the tube, and a very small particle of iodine is introduced, and vaporized by the application of a gentle heat. The iodine vapor combines with the mercury, and the gray ring is in this manner converted into mercuric iodide, which is yellow while hot, but changes to red on cooling; the change in color is sometimes quite slow, but may be brought about instantly by touching the yellow deposit with a hard body, such as a glass rod or a copper wire.

§ 252. The mercurial ring in another tube, from which the copper has been removed, is dissolved in nitro-hydrochloric acid. A solution of mercuric chloride is thus obtained. This solution is evaporated to dryness,

and the residue is dissolved in a little water; the following tests are then applied:—

a) A drop of stannous chloride added to part of this solution will produce a white precipitate of mercurous chloride, which changes to gray on the addition of more stannous chloride.

b) Hydrogen sulphide gives a precipitate which is at first white, but changes to yellowish and black, if the reagent be not employed in very small quantity. Ammonium sulphide acts in the same manner in mercurial solutions.

c) Ammonia produces a white precipitate of mercurammonium chloride.

§ 253. The dry residues of mercuric chloride obtained from other tubes in the same manner, may be tested in white porcelain dishes by the following reagents.

a) A drop of potassium iodide solution produces a red color, due to the formation of mercuric iodide; the color disappears in an excess of the reagent, in which mercuric iodide is soluble.

b) Potassium hydrate gives a yellow, or orange-colored spot of mercuric oxide, which is dissolved with difficulty by a large excess of the reagent.

DESTRUCTION OF THE ORGANIC MATTER.

§ 254. In whatever form, excepting cinnabar, mercury may have been present in the original mixture, it is always obtained in solution as mercuric chloride by the process about to be described. Cinnabar is not poisonous, and is, therefore, not likely to be encountered in a toxicological investigation; but were it present, a sufficient quantity of it would be dissolved to indicate the presence of mercury, though the larger portion of it would remain unaffected.

As all mercurial compounds are volatile, the organic matter present may not be destroyed by any process which depends upon the application of a high degree of heat, such as deflagration with potassium nitrate.

The most satisfactory and best method consists in the use of hydrochloric acid and potassium chlorate, pre-

cisely as has been indicated in the case of arsenic (§ 236). After all of the organic matter has disappeared, and the liquid is fit for filtration, it is heated to expel the excess of chlorine, and filtered hot. The filtrate is then saturated with hydrogen sulphide, and the black precipitate formed is collected on a filter, and thoroughly washed with distilled water. It is insoluble in either hydrochloric or nitric acid separately, but dissolves readily in a mixture of the two. It is, therefore, at once dissolved in nitro-hydrochloric acid, the solution is evaporated to dryness, and the residue is dissolved in a small quantity of water, acidulated with a few drops of hydrochloric acid to facilitate the solution of any mercuric sulphate which might have been formed.

When introduced into Marsh's apparatus, this solution does not affect the character of the gas evolved, and no spots or rings can be obtained. The solution is subjected to the tests given in sections 250-253.

Should sufficient of the dry residue from the evaporation of the solution in nitro-hydrochloric acid be available, a portion of it may be mixed with some dry sodium carbonate, and the mixture strongly heated in a small reduction tube. The mercuric salt will thus be reduced, and metallic mercury will volatilize and condense on the sides of the tube. The mercury rings may be tested according to section 251.

In addition to the characters of these rings that have already been mentioned, they differ from arsenical and antimonial rings in the following particulars:—

The mercurial rings sublime without odor.

They are not oxidized by heating in the presence of air.

They are not dissolved by an alkaline solution of sodium hypochlorite.

Ammonium sulphide converts them into black, mercuric sulphide.

ELECTROLYTIC TEST.

§ 255. Should the presence of mere traces of mercury be suspected, it is not advisable to precipitate by hydro-

gen sulphide the solution obtained after destroying the organic matter by potassium chlorate. In such a case, the best course is to directly submit the filtered liquid to electrolysis, a battery of about five Smee cells being employed. The positive pole should consist of a small platinum plate, while a gold wire, one or two millimetres in diameter, and two or three centimetres long, forms the negative electrode. After some time—two or three days if the amount of mercury present be very small—all of the mercury is removed from the solution and deposited on the gold wire, where its presence is indicated by its color. This color should not, however, be relied upon as conclusive, but the wire should be heated in a small tube, and the usual tests applied to the mercurial sublimate so obtained.

§ 256. Since mercury is largely employed as a remedial agent, a quantitative estimation of that extracted from the organs or contents of the intestinal canal in cases of poisoning, is often highly important. For this purpose the preceding electrolytic method is quite satisfactory; the gold wire is weighed before the operation, and again after the deposition of mercury is complete, and before heating in the tube. The increase in weight of course indicates the quantity of mercury present. 100 parts of mercury correspond to 117.25 parts of calomel, or to 135.5 parts of corrosive sublimate.

LEAD.

§ 257. Cases of acute poisoning by compounds of lead are of comparatively rare occurrence, and this may be understood if the unpleasant, astringent taste of these compounds, and the large dose required to produce dangerous effects, be borne in mind. On the other hand, preparations of lead are so largely employed in the arts, and the metal itself serves such a variety of purposes, that cases of chronic lead-poisoning are by no means infrequently encountered. The metal or one of its compounds may be inhaled in the form of dust, in printing offices, white-lead and minium factories, and paint shops.

Lead is readily acted on by many chemical agents; even pure aerated water will dissolve a notable quantity of lead, forming a hydrate, and the use of such water, after passing through lead pipes, for drinking or culinary purposes, may be followed by lead-poisoning. If the water contain carbonates or sulphates, even in small proportion, as do most river and spring waters, the surface of the metal soon becomes covered with a thin but insoluble crust of lead carbonate or sulphate, and this protects the metal from further corrosion. Hence leaden conduits may be safely employed for river waters, while the use of lead cisterns and pipes for the storage and conveyance of rain-water is highly dangerous.

The glazing of common pottery and earthenware often consists of a highly basic silicate of lead. This is readily acted on, not only by acids, but also by pure aerated water, and the use of vessels so glazed for culinary purposes has frequently given rise to lead-poisoning.

DETECTION IN ORGANIC MIXTURES.

§ 258. If a solution suspected to contain lead be perfectly clear, it needs no preparation, and hydrogen sulphide is passed through it immediately, and until the liquid smells strongly of the gas. A black precipitate indicates the presence of lead. This precipitate, which

is lead sulphide, is collected on a filter, and treated as directed farther on (§ 261). If the substance to be examined consist of both solid and liquid, the latter may be separated by filtration, and at once precipitated by hydrogen sulphide, the residue on the filter being examined separately, or the organic matter may be destroyed, and the entire mass treated simultaneously.

§ 259. *Destruction of organic matter.*—If the examination of the liquid portion should fail in disclosing the presence of lead, it should not be at once concluded that none is present, for the metal may be contained in combination with organic matter, or in some other insoluble form, in the solid or semi-solid matters left on the filter. This matter should, therefore, be mixed with sodium hydrate and ammonium nitrate, in suitable proportions, and the mixture evaporated to dryness, after which the residue is fused in a clay or porcelain crucible. Any lead present is thus converted into nitrate or nitrite. The residue is powdered, and dissolved in water acidulated with nitric acid; the solution obtained is ready for precipitation by hydrogen sulphide.

§ 260. When solid organic matters, such as animal tissues, are to be examined for the presence of lead, the process described in § 236 may be employed. The mixture of hydrochloric acid and potassium chlorate will dissolve all of the lead compounds, even the metal itself. The lead chloride thus formed will remain in solution while the mixture is hot, but is usually partially precipitated on cooling; by filtering the liquid at the boiling point, nearly if not quite all of the lead will pass into the filtrate, which is then immediately submitted to the action of hydrogen sulphide. Since some lead chloride may remain on the filter, the latter with its contents is treated as directed in § 259.

§ 261. The precipitate formed by hydrogen sulphide is black when the gas has been passed through the solution for a sufficient time, but may have a red color, due to a sulpho-chloride of lead, immediately when first formed. It is collected on a filter, and the filtration and washing are conducted as rapidly as possible, because

moist lead sulphide becomes oxidized to sulphate more or less quickly on contact with the air.

This precipitate is insoluble in either ammonia, ammonium carbonate, or ammonium sulphide. It is only slightly soluble in hydrochloric acid, readily in nitric acid.

It is dissolved in boiling dilute nitric acid, lead nitrate being formed, but some insoluble lead sulphate is always formed at the same time; this is separated by filtration, and tested as directed in § 262, *a*.

§ 262. The clear filtrate is then mixed with a little ammonium nitrate, the mixture is evaporated to dryness, and the residue calcined in a porcelain crucible. When cold, the mass so obtained is boiled with water acidulated with a little nitric acid, and the solution is submitted to the following tests:—

a) Sulphuric acid and solutions of the soluble sulphates produce a white precipitate of lead sulphate, distinguished by the following characters: it dissolves in potassium hydrate, in hydrochloric acid, tartaric acid, and in a boiling solution of ammonium acetate. It is colored black by hydrogen sulphide, and yellow by potassium chromate.

b) Hydrochloric acid and solutions of the soluble chlorides produce a white precipitate, which redissolves when the liquid is boiled, but is again precipitated in brilliant crystalline scales as the solution cools. This precipitate is insoluble in ammonia, and unaffected by that reagent. When it is mixed with dry sodium carbonate and heated in the inner blowpipe flame, a small globule of metallic lead is obtained; this globule is malleable, and may be easily flattened by the blow of a hammer. If it be heated in the outer blowpipe flame, it becomes oxidized, and produces a reddish-yellow incrustation on the charcoal. These blowpipe reactions are common to all compounds of lead.

c) Potassium chromate gives a yellow precipitate of lead chromate, soluble in potassium hydrate.

d) Potassium iodide produces a bright yellow precipitate, soluble by the aid of heat, but again deposited in glittering, golden-yellow scales on cooling.

e) Ammonia throws down a white precipitate, insoluble in excess.

f) Potassium and sodium hydrate yield white precipitates, soluble in an excess of the reagent.

Examination of Water suspected to contain Lead.

§ 263. In cases of chronic lead-poisoning, the lead may often be traced to the water employed. The latter may contain sufficient of the metal to yield a more or less perceptible precipitate with hydrogen sulphide without concentration; however, the analysis is always more easily made by evaporating the water to one-twentieth, or even one-fiftieth, of its volume, in a small porcelain capsule. It may be necessary to evaporate five or ten litres of the water, and in such a case small portions should be evaporated at a time, in the same capsule. The residue is dissolved in concentrated nitric acid, the solution evaporated to dryness, and the residue, which consists principally of sulphates and nitrates, is treated with distilled water, and the solution filtered. The filtrate is subjected to the tests indicated in § 262. The residue on the filter may contain lead sulphate, and is tested according to § 262, *a*.

QUANTITATIVE ESTIMATION.

§ 264. The quantity of lead present may be most rapidly determined by converting the metal into sulphate, and weighing the latter. The precipitate of lead sulphide is boiled with concentrated nitric acid, the liquid is evaporated to dryness, and the residue is treated with concentrated sulphuric acid, and again evaporated, in order to expel all of the nitric acid. The temperature must finally be raised until no more vapors of sulphuric acid are disengaged. The whole operation, including the weighing, may be conducted in a tared porcelain crucible. 100 parts of lead sulphate contain 68.3 parts of lead.

COPPER.

§ 265. Like lead, copper is not often employed for the purpose of criminally destroying life, but it is not unfrequently introduced into the system accidentally in articles of food, with serious and sometimes fatal results. The principal cause of such accidents is the use of untinned copper vessels for culinary purposes; and although such vessels, when perfectly clean, may be used without danger in the preparation of certain articles of food, the number of alimentary substances capable of acting upon and dissolving small quantities of the metal is so great, that it is far safer to avoid the use of untinned copper vessels in all culinary operations. Acid and fatty substances especially, and liquids containing common salt and other saline matters in solution, should never be boiled in such vessels, since the quantity of copper dissolved by them is sometimes so considerable as to impart a green or bluish color to the mixture.

Imperfectly tinned copper vessels are, in this respect, more dangerous than such as are entirely untinned, because the exposed portions of the copper are more rapidly acted on, by the electrolytic influence of the tin, than were no tin present.

§ 266. It seems that small quantities of copper are very often, if not always, normally present in the human body especially in the liver. Therefore, in a toxicological investigation, it is of importance to determine the quantity of this metal that can be extracted from the liver, as a mere trace might not be abnormal.

DETECTION IN ORGANIC MIXTURES.

§ 267. In mixed animal or other substances, copper may exist in solution, or in combination with organic matters, or in some other form which is more or less insoluble in water. For this reason, when the mixture to be examined consists of both solid and liquid matters, it should first be warmed with a little hydrochloric or

acetic acid, by which the copper will be brought into solution. The liquid may then be filtered from the insoluble portion, which should be retained for subsequent examination (§ 268), or the whole of the organic matter may be at once destroyed, as may be selected.

A decisive preliminary test may be made upon the filtrate, thus: a few drops of the solution are placed upon a bright iron knife-blade, or any other polished iron surface, when, should any copper be present, it will be deposited in the metallic state on the iron, and may readily be recognized by its red color. If desired, a needle or knife-blade may be immersed in the liquid, and will soon become covered with a film of copper: but as a quantitative estimation may be necessary, it is more advisable to make the preliminary test upon as small a quantity of the liquid as possible, adding the remainder to the solution obtained as described in the next section, or making a separate quantitative determination of the copper it contains, as will shortly be described.

§ 268. *Destruction of the Organic Matter.*—The residue left upon the filter, together with any solid substances, such as animal tissues, is finely divided and treated with hydrochloric acid and potassium chlorate as directed in § 236. The liquid thus obtained is boiled and filtered; if desired, a preliminary test may be made upon the filtrate by placing a few drops of it on a clean knife-blade. A current of hydrogen sulphide is then passed through the clear solution, until it is completely saturated with the gas. If any copper be present, it will be thrown down as a black precipitate of cupric sulphide. This precipitate has a great tendency to become oxidized, and must therefore be separated by rapid filtration, and quickly washed with water that has been boiled to expel air, and which contains a little hydrogen-sulphide.

§ 269. The precipitate is dissolved in nitric acid, and the copper thus converted into cupric nitrate; the solution is greenish-blue, and may still contain organic matter which had been thrown down with the cupric sulphide. It is therefore made strongly acid, some ammonium nitrate is added, and the whole is evaporated to

dryness; the residue is calcined until all of the organic matter is destroyed, and is then dissolved in water slightly acidulated with nitric acid. The solution is submitted to the following tests:—

a) Ammonia produces a bluish-white precipitate, which dissolves in an excess of the reagent, forming a dark-blue solution.

b) Potassium ferrocyanide gives a mahogany-colored precipitate, even in very dilute solutions of copper. Mere traces of the latter metal produce only a reddish-brown color, which can be best seen by holding the test-tube in front of a sheet of white paper, or other white surface. The liquid to which this test is applied must be acid, since the precipitate is soluble in ammonia and the alkaline hydrates.

c) A drop of the solution placed upon a clean iron surface will deposit a film of metallic copper.

d) Potassium or sodium hydrate will throw down a pale-blue precipitate of cupric hydrate, which becomes converted into black, anhydrous cupric oxide when boiled. If some glucose be added to the cupric solution before the alkaline hydrate, no precipitate is formed on the addition of the latter, but on boiling the solution, cuprous oxide, which is red, is immediately thrown down.

e) If the solution obtained by dissolving the precipitated cupric sulphide in nitric acid be introduced into a platinum crucible or capsule, which is connected with the negative pole of a voltaic battery, while a platinum plate suspended in the liquid in the crucible constitutes the positive pole, in the course of several hours, according to the strength of the electric current, all of the copper will be removed from the solution and deposited on the interior of the platinum vessel. This test is very delicate, and may be applied to the quantitative estimation of copper, the weight of the crucible without the deposit of copper being known. The copper may be dissolved from the platinum vessel by nitric acid, and the preceding tests made subsequent to the quantitative determination, if so desired.

Quantitative Estimation.

§ 270. Copper is usually estimated in the form of cupric oxide, CuO . Should organic matter have been destroyed by hydrochloric acid and potassium chlorate, the precipitated sulphide is dissolved in nitric acid, and any remaining traces of organic matter are destroyed as directed in § 269. The residue is dissolved in dilute nitric acid, and the solution is again precipitated by hydrogen sulphide. The precipitate is redissolved in nitric acid; the solution is evaporated to dryness in a covered porcelain crucible, and the residue is gradually heated to bright-redness. The cupric oxide thus formed may contain some cuprous oxide; it is therefore moistened with concentrated nitric acid, again calcined, and after cooling is ready for weighing.

Ashes, and the residue from the deflagration of organic matter, are exhausted with nitric acid, the solution is precipitated by hydrogen sulphide, and the operation continued as just described. 100 parts of cupric oxide correspond to 79.87 parts of metallic copper.

Copper may also be separated in the metallic state by electrolysis, and weighed directly, as mentioned in the preceding section, should no other metal be present which would be deposited at the same time.

ZINC.

§ 271. Accidents have occurred from the unintentional introduction of compounds of zinc into the system, and zinc sulphate has sometimes been administered with criminal intentions. Besides this, zinc sulphate, or *white vitriol*, as it is commonly called, is often given as an emetic in cases of poisoning, so that it may be encountered in toxicological examinations, and the analyst must be familiar with its reactions.

The destruction of organic matter is accomplished by hydrochloric acid and potassium chlorate, and the mixture is filtered as directed in § 236. Zinc is not precipitated by hydrogen sulphide from solutions containing free mineral acids; therefore, hydrogen sulphide is first passed through the filtrate, and any precipitate that may form is separated by filtration, and examined for such other metals as may be indicated by its color.

An excess of ammonia is then added to the filtrate, and should any precipitate be formed, the liquid is again filtered. Hydrogen sulphide is passed through the clear solution, and if zinc be present a white precipitate of zinc sulphide will be thrown down.

It is collected on a filter, and rapidly washed with tolerably concentrated acetic acid, after which it is dissolved in a little nitric acid; the solution so obtained is evaporated to dryness, and the residue calcined and dissolved in water acidulated with nitric acid. The solution of zinc nitrate is filtered, if necessary, and tested as follows:—

a) Add sufficient sodium acetate to react with the zinc nitrate and nitric acid, and replace all of the free nitric acid by acetic acid. Hydrogen sulphide or ammonium sulphide will then produce a white precipitate of zinc sulphide.

b) Potassium ferrocyanide produces a white precipitate, insoluble in dilute acids, but soluble in potassium hydrate, by the aid of heat, and reprecipitated from this solution on the addition of hydrochloric acid.

c) Potassium or sodium carbonate gives a white precipitate of basic zinc carbonate, insoluble in an excess of the reagent. If this precipitate be boiled in the liquid in which it was formed, and then collected on a filter, washed, and dried, it may be ignited in a porcelain crucible, and will be converted into zinc oxide, which is yellow while hot, and white when cold.

DETECTION OF ACIDS.

§ 272. Certain acids only act as poisons when they are introduced into the stomach in a concentrated state; such are the mineral acids, sulphuric, hydrochloric, and nitric. The compounds of these acids with bases that are not themselves poisonous, may often be safely ingested in comparatively large doses, and even dilute solutions of the acids may sometimes be freely taken into the stomach without ill effects. Other acids, such as tartaric and citric, may be safely administered in a concentrated state, in small doses, and produce poisonous effects only when taken in large quantities. On the other hand, some acids are always poisonous, in whatever quantity or state of concentration they may be taken; their soluble salts, and some of their insoluble salts, also possess poisonous properties. Notable examples are oxalic and hydrocyanic acids.

The three principal mineral acids, sulphuric, hydrochloric, and nitric, have frequently been employed as criminal poisons and as means of suicide, and it is necessary that the medical chemist shall be able to identify them, either mixed with organic matters, such as the contents of the stomach, or vomited matters, or in the stains which these acids produce on clothing or other fabrics.

Generally, the highly acid reaction of the material under examination is a sufficient index to the nature of the poison, and the task of the analyst is facilitated;

but in case an alkaline antidote, such as an alkaline carbonate, magnesia, or chalk, has been administered, it becomes necessary to seek for the acid in a saline combination. An aqueous extract of the matters may then be first examined, and the manner in which this responds to the tests will usually be sufficient to distinguish between the sulphates and chlorides which would naturally be expected to be present, and the large excess of these salts that would indicate a probable poisoning.

§ 273. The following method of procedure, proposed by Roussin, may be relied upon for the detection of the three acids in question when they are present in the free state :—

The liquid portion of the matter is separated by filtration, and the solid parts are washed with water, which is then united with the filtrate. The latter is evaporated to dryness, in a retort provided with a receiver, and heated to a temperature not above 110° , on an oil-bath.

a) Nitric acid, if present, produces red fumes towards the end of the operation, and the distilled liquid will give a rose or brown tint when mixed with a concentrated solution of ferrous sulphate and strong sulphuric acid.

b) Sulphuric acid will react with the organic matter, and be reduced to sulphurous acid, which can be detected in the distillate. In this case, the residue in the retort becomes charred and blackened.

c) Hydrochloric acid will pass into the distillate, and may be detected by the addition of silver nitrate. As a small quantity of hydrochloric acid is obtained by distilling the normal contents of the stomach, unless the precipitate of silver chloride be somewhat abundant, it must not be too hastily decided that hydrochloric acid in poisonous quantity was present. In such a case, it is necessary to make a quantitative estimation.

d) Oxalic acid, if present, would remain in the retort, the contents of which would not be blackened. The residue is treated with alcohol, and the liquid so obtained is filtered, and tested with calcium acetate, with which oxalic acid forms a white precipitate of calcium oxalate, soluble in mineral acids, especially hydrochloric, but insoluble in acetic acid.

Sulphuric Acid.

§ 274. The presence of free sulphuric acid may be easily detected, even when mixed with a large proportion of organic matter. If the mixture be viscid or semi-solid, it is diluted with water to such a consistence that it may be filtered. Solution of barium chloride is added to the filtrate, and, if sulphuric acid be present, a white precipitate of barium sulphate is thrown down, and this precipitate is insoluble in nitric acid, even by the aid of boiling. It is collected on a filter, dried, mixed with an excess of powdered charcoal, and heated to whiteness in a small porcelain crucible. Barium sulphate is thus reduced to barium sulphide, which will disengage hydrogen sulphide when moistened with hydrochloric acid.

Sulphuric acid, whether free or in combination, is readily detected in this manner. Should the acid have been partially neutralized by the administration of an antidote, the presence of small quantities of free acid may be detected as directed in § 273. However, it is not often that any serious uncertainty can exist as to whether the sulphuric acid found mixed with organic matter was or was not uncombined, especially in cases of suspected poisoning, since the corrosive effects of the acid upon the parts with which it has come in contact, or other corroborative circumstances, will generally of themselves furnish evidence sufficiently conclusive.

§ 275. The blue liquid commonly known as *sulphate of indigo*, has frequently been criminally employed by poisoners and by suicides. It is made by dissolving indigo in fuming sulphuric acid, and diluting the solution with water, and is used by dyers, and sometimes as a washing-blue. The deep blue color of the substances under examination leads the analyst to suspect the presence of indigo, and the latter is easily characterized by the reddish-yellow color which it assumes when heated with nitric acid. When sulphate of indigo is boiled with nitric acid, the indigo is converted into a substance called isatine, and sulphuric acid may then be detected in the solution by means of barium chloride, as previously described. Indigo is transformed into white-indigo when

boiled with glucose and milk of lime, but the blue color is gradually restored by contact with the air.

Hydrochloric Acid.

§ 276. Since chlorides may always be expected to be naturally present in such mixtures as the contents of the stomach and vomited matters, it is of little service to apply tests directly to the clear portions of such mixtures. Besides this, silver nitrate, which is used in testing for hydrochloric acid and chlorides, precipitates a great number of organic substances, and these must therefore be eliminated before applying the test. This is accomplished, as indicated in § 273, by distilling to dryness the liquids to be examined, and testing only the portion which distils. All of the free hydrochloric acid will be found in the distillate, and could only be mistaken for a few other volatile acids, from which subsequent tests will at once distinguish it.

If hydrochloric acid be present, the contents of the receiver will be acid, and will produce a white, curdy precipitate with solution of silver nitrate: this precipitate consists of silver chloride; it is insoluble in nitric acid, but dissolves readily in ammonia. On exposure to light, it rapidly darkens in color, and becomes violet or bluish-black.

§ 277. QUANTITATIVE ESTIMATION.—As has already been mentioned, hydrochloric acid is always obtained by distilling matters which may normally be found in the stomach, since free hydrochloric acid exists in the gastric juice. The quantity of the acid derived from this source is, however, so small that it may readily be distinguished from the comparatively large quantity usually to be found when the acid has been swallowed. Such distinction must be made by a quantitative analysis.

The silver chloride obtained by precipitating the distilled liquid by an excess of silver nitrate is collected on a filter and thoroughly washed with distilled water; after drying, the filter is burned separately, and both ash and precipitate are heated to redness in a tared porcelain

crucible. It is then weighed; 100 parts of silver chloride correspond to 25.43 parts of hydrochloric acid.

It is well also to distil a mixture similar to that suspected to contain hydrochloric acid, and to estimate the amount of hydrochloric acid in the filtrate. A direct comparison may then be made between the quantity found in the suspected mixture, and that which might be expected to be normally present.

Nitric Acid.

§ 278. As nitric acid only exists in inappreciable quantities, if at all, in the substances such as vomited matters, the digestive organs and urine, usually submitted to toxicological examination, the presence of the smallest trace of this body may be regarded as abnormal. Again, nitric acid poisoning may usually be recognized without difficulty by the peculiar yellow stains which the acid imparts to the epidermic tissue; this coloration, however, is not always well marked on the mucous membrane of the digestive organs.

The chemical analysis is made upon an aqueous extract of the matters. This is filtered, neutralized with potassium carbonate, and evaporated to dryness on a water-bath. The residue will contain potassium nitrate, which may be deposited in a crystalline form, if there be not too much organic matter present. It is redissolved in the smallest possible quantity of water, and the solution is filtered, and tested as follows:—

a) A portion of the filtrate is gently heated in a test-tube with a small piece of copper wire or foil and strong sulphuric acid. If nitric acid be present, orange-colored vapors will be disengaged.

b) Another portion is mixed with a solution of ferrous sulphate, and poured, without mixing, upon strong sulphuric acid, in a test-tube. The presence of nitric acid will then be indicated by a rose-colored or brown ring at the surface of contact of the two liquids. It should be ascertained that the color is not produced by the suspected solution and sulphuric acid alone, as might be the case should certain organic substances be present.

c) A third portion is treated with an excess of potassium hydrate, and evaporated to dryness. Ammonia may be given off, in which case the residue is heated on the water-bath until no more ammoniacal odors are perceptible; a second treatment with potassium hydrate may be necessary to expel all of the ammonia. The residue is then dissolved in about four times its volume of water, and heated with platinized zinc, or with aluminium wire or filings. Should nitric acid be present, it will be reduced by the nascent hydrogen; ammonia will be disengaged, and may be recognized by its odor and by the white fumes which are produced when a rod moistened with hydrochloric acid is held near the mixture.

d) A fourth portion of the solution is mixed with about its own volume of concentrated sulphuric acid, a few drops of sulphate of indigo are added, and the mixture is heated. If nitric acid be present, the indigo will be decolorized. This reaction is hardly applicable when the suspected solution has a dark color.

Detection of Mineral Acids in Stains on Clothing.

§ 279. Should the stains be quite recent, the acid may usually be detected by cutting out the stained part, boiling it with water, and testing the solution, as has been indicated, for the several acids.

Sulphuric acid stains are generally somewhat moist, and have a brown or red color, but the color varies with the nature of the material and the dye. They nearly always disappear when moistened with ammonia.

Owing to the volatile nature of hydrochloric acid, it is often impossible to satisfactorily prove its presence in a stain.

Nitric acid stains are usually brown or yellowish, and, unlike those caused by sulphuric acid, soon become dry, and destroy the fabric. They are not modified by alcohol, ether, or benzol, but assume an orange color when moistened with potassium hydrate or ammonia.

Oxalic Acid.

§ 280. Oxalic acid may be separated from organic mixtures by evaporating the latter to dryness, and extracting the residue with alcohol acidulated with hydrochloric acid. It may be necessary to perform this extraction twice, and should lime or magnesia have been administered as an antidote, so that an insoluble oxalate is present, the residue from the alcoholic extraction must be boiled with dilute hydrochloric acid, which, after filtration, is added to the alcoholic extract. The latter is then evaporated, and after the alcohol has been expelled, the aqueous solution is filtered, if necessary, and tested by the following reactions.

a) A portion of the liquid is exactly neutralized with ammonia, and lime-water or solution of calcium sulphate is added: oxalic acid will occasion the formation of a white precipitate of calcium oxalate, insoluble in acetic acid, but soluble in hydrochloric acid, and reprecipitated from this solution on the addition of ammonia. If this precipitate be collected, washed, dried, and calcined at a high temperature, it will leave a residue of lime, which will restore the blue color to moistened red litmus paper, and change to brown the yellow color of turmeric.

b) Lead acetate or subacetate throws down a white precipitate of lead oxalate. This reaction may be applied to the separation of oxalic acid from organic mixtures: a solution of lead acetate is added to the mixture as long as it produces any precipitate; the lead oxalate thrown down is collected on a filter, well washed, suspended in water, and decomposed by a stream of hydrogen sulphide. Lead sulphide is formed, and is deposited, together with most of the organic matter which was precipitated with the lead oxalate, while oxalic acid enters into solution and is separated by filtration. When the clear filtrate is sufficiently evaporated, the acid is deposited in small, needle-like crystals, which are generally almost colorless.

c) Silver nitrate produces a white precipitate of silver oxalate in solutions containing oxalic acid or an oxalate. If this precipitate be dried and gently heated on a piece

of platinum foil, it becomes brown or black, and decomposes with a slight explosion.

§ 281. QUANTITATIVE ESTIMATION.—In order to determine the quantity of oxalic acid in a liquid containing the free acid or one of its salts, the solution is acidulated with acetic acid, and precipitated by calcium chloride; the mixture is then boiled and filtered. The precipitate left on the filter is washed, dried, and ignited in a tared crucible. After cooling, the residue is moistened with a saturated solution of ammonium carbonate, and again heated to incipient redness. All of the calcium oxalate is thus converted into calcium carbonate, which may now be weighed. 100 parts of calcium carbonate correspond to 126 parts of crystallized oxalic acid.

HYDROCYANIC ACID (PRUSSIC ACID).

§ 282. Hydrocyanic acid and its soluble compounds are frequently the agents of accidental and criminal poisoning. The poisonous nature of the cyanides is due to the liberation of hydrocyanic acid by the action of the acids contained in the gastric juice. The presence of this acid may usually be detected, even when very much diluted, by its peculiar smell which recalls that of crushed peach-kernels. Hydrocyanic acid is very volatile, and is at the same time quite unstable, and soon decomposes, especially in the presence of organic matter, so that unless the chemical examination be made shortly after the suspected poisoning, all traces of the acid may have disappeared, although comparatively large quantities were originally present.

DETECTION OF HYDROCYANIC ACID VAPORS.

§ 283. The following tests may be made directly upon the suspected substance, and are not invalidated by the presence of organic matters or other foreign compounds:

- a) A portion of the suspected matter is acidulated, if

neutral or alkaline, with dilute sulphuric acid and placed in a watch-glass, over which is inverted another similar watch-glass in which a drop of a solution of silver nitrate has previously been placed. The silver nitrate solution must not be allowed to flow into the lower glass. This test may also be made by inverting a watch-glass containing a drop of silver nitrate over the jar in which the suspected substance has originally been placed. If hydrocyanic acid be present, the silver nitrate solution will soon become turbid, and the reaction may be hastened by warming the lower vessel. The turbidity is caused by the formation of silver cyanide; the precipitate does not change color on exposure to light, and is soluble in hot nitric acid; it is thus distinguished from silver chloride, which would be formed were any free hydrochloric acid present in the suspected mixture. If this test yield affirmative results, all of the following tests should be applied; but, if the results be negative, other tests applied directly for the detection of the vapor will probably be fruitless.

b) A watch-glass holding some of the suspected substance, or the vessel originally containing it, has inverted over it, as in the preceding experiment, a watch-glass in which a drop or two of a solution of potassium hydrate has been placed. On warming the lower glass, hydrocyanic acid will volatilize, and, reacting with the potassium hydrate, will form potassium cyanide. After the lapse of five or ten minutes, the upper glass is removed, and the spot is touched with a drop of ferrous sulphate and a drop of ferric chloride; one or two drops of dilute hydrochloric acid are then added, when, should hydrocyanic acid have been present, a blue precipitate (Prussian blue) will be formed. This test is usually known as the *iron test*, and is without fallacy, but demands care in its manipulations.

c) *Liebig's test* is made in a similar manner, but the upper watch-glass contains only a drop of yellow ammonium sulphide. If hydrocyanic acid be present, the ammonium sulphide will soon become colorless, and when this is accomplished, which may require fifteen minutes or half an hour, the upper glass is removed; it will con-

tain ammonium sulphocyanate, formed by the reaction of the hydrocyanic acid and ammonium sulphide, and when this is touched with a drop of ferric chloride a blood-red color is produced. It is sometimes recommended to evaporate the ammonium sulphocyanate to dryness before adding the ferric chloride, but, if the yellow ammonium sulphide be completely decolorized, this is not necessary. The red color is bleached by solution of mercuric nitrate, and is thus distinguished from that which might possibly be produced under similar circumstances by acetic acid.

DETECTION OF HYDROCYANIC ACID IN SOLUTION.

§ 284. The mixture of organic matter or other substance suspected to contain hydrocyanic acid, is, if neutral or alkaline, acidulated with dilute sulphuric acid, and introduced into a retort, the neck of which is slightly inclined upwards, and in communication with a Liebig's condenser inclined downwards. The retort is then heated in an oil-bath to a temperature not above 110° , and the contents are distilled until about one-eighth of the liquid has passed over. The distillate is submitted to fractional distillation, collecting about 3 c.c. for every 100 c.c. of liquid, and changing the recipient between every 3 c.c. Hydrocyanic acid, if present, will be found in the first fractions, and, if in any quantity, may be recognized by its odor.

The distilled liquid is tested as follows:—

a) Silver nitrate produces a white precipitate, soluble in ammonia and hot nitric acid. This precipitate is collected on a filter, washed, and dried; if a sufficient quantity of it be obtained, a portion may be heated in a small tube; cyanogen gas will be disengaged, and when lighted will burn at the mouth of the tube with a purple flame, while a black residue remains in the tube.

Place a very small fragment of iodine at the bottom of a small tube closed at one end, and above it as much as can be spared of the supposed silver cyanide. Apply a very gentle heat, by holding the tube at some distance above a flame, when cyanogen iodide will be formed, and will condense in the cool part of the tube in fine, white

needles. If very little silver cyanide be used, it is well to cover it with a layer of sodium carbonate, to retain any excess of iodine. Cut off that portion of the tube which contains the sublimate, and warm it in a test-tube with a little dilute ammonium sulphide, in which it will dissolve. Evaporate the solution to dryness in a porcelain capsule on a water-bath, and treat the residue with a drop of ferric chloride; a blood-red color will be produced.

b) Add to some of the distilled liquid a solution of potassium hydrate, and then a few drops of ferrous sulphate and a drop of ferric chloride, and boil. After cooling, add a slight excess of dilute hydrochloric acid. If the liquid contained hydrocyanic acid, a blue precipitate of ferric ferrocyanide (Prussian blue) is formed, either immediately or after standing for a few hours.

c) Another portion of the distillate is evaporated to dryness with a few drops of yellow ammonium sulphide (or if the latter be completely decolorized after boiling, the evaporation is not necessary), and tested with a few drops of ferric chloride. Hydrocyanic acid will thus have been converted into ammonium sulphocyanate, and this produces a blood-red color with ferric salts.

QUANTITATIVE ESTIMATION.

§ 285. A known weight of the substance is distilled, as has already been described, and the distillate is rectified over borax, in order to remove all traces of hydrochloric acid which might be present. The distilled liquid is precipitated by an excess of silver nitrate, and the silver cyanide thrown down is collected on a tared filter, carefully washed, and dried at 100° . It is then weighed in the filter, and the weight of the latter deducted. 100 parts of silver cyanide correspond to 20.15 parts of anhydrous hydrocyanic acid.

PHOSPHORUS.

§ 286. The almost universal use of phosphorus matches, the ease with which rat poisons containing phosphorus may be obtained, the general knowledge of the poisonous properties of these preparations, and the small dose required to produce death, have combined to render phosphorus one of the most common agents of criminal and accidental poisoning.

If the substance to be examined has not been long exposed to the air, it may contain phosphorus in the free state; but in many cases, oxidation will have converted the phosphorus into phosphorous acid or phosphoric acid. Since this last is a normal constituent of the body and of the food, it would afford no evidence of the administration of phosphorus, but it may be considered as proven that in most cases phosphorus may be detected after several weeks' time, in a body on which no previous autopsy has been made.

If possible, the vomited matters should be examined, as they have been found to contain the largest proportion of phosphorus; but unless this examination be made promptly, all of the poison may be oxidized. The oxidation or non-oxidation of phosphorus in such matters depends on various circumstances, almost impossible to understand or foresee: in some cases the poison has been found unaltered after the lapse of several months, in others it has been wholly oxidized within two or three days.

The organic matter should first be examined as to its odor, that of phosphorus being very characteristic, and as to its luminosity in the dark. It should be also ascertained whether any solid particles of the phosphorus compound can be detected mechanically.

§ 287. The suspected matter is then acidulated with dilute sulphuric acid, a little water is added, if necessary, and the mixture is introduced into a glass flask. By means of a glass tube, two or three centimetres in diameter and fifty or sixty centimetres long, bent twice at right

angles, the flask is connected with a vertical Liebig's condenser, made wholly of glass. The apparatus is placed in a perfectly dark room, and the liquid in the flask is heated: as soon as it enters into ebullition, if phosphorus be present, luminous vapors will appear in the flask, and gradually extend into the tube, becoming permanent at the point at which the aqueous vapor begins to condense. The appearance of luminous vapors is characteristic of the presence of free phosphorus, and should any quantity of the latter be present, it will be found in the form of minute globules in the distilled liquid. This process, suggested by Mitscherlich, is exceedingly delicate.

§ 288. Fresenius and Neubauer combine the method of Mitscherlich with one which was suggested by Dussard and Blondlot. After the phosphorescence has ceased in the flask and tube, a solution of silver nitrate is added to the distilled liquid, and the distillation is continued for some time longer. The precipitate formed by the silver nitrate is collected, well washed, and introduced into a capacious apparatus for generating hydrogen by means of zinc and dilute sulphuric acid. The stream of gas evolved is freed from all traces of hydrogen sulphide by being passed through a U tube filled with pumice-stone impregnated with potassium hydrate, and is burned at a platinum jet. Under these circumstances, pure hydrogen will burn with an almost invisible flame, but the presence of phosphorus communicates a characteristic greenish color to the flame.

After the detection of phosphorus by this colored flame, the gas may be passed into a solution of silver nitrate, where it will occasion a dark precipitate of silver phosphide; this is collected, washed, boiled with a little strong nitric acid, and the solution is evaporated to dryness. On adding a little water, and a drop of very dilute ammonia, a yellow color or precipitate of silver phosphate will be formed.

DETECTION OF ALKALOIDS IN ORGANIC MIXTURES.

§ 289. The active principles of most vegetable poisons may generally be recognized without great difficulty when in a pure state, but when they exist in complex organic mixtures their detection often becomes one of the most trying problems of the toxicological expert. The foreign matter present cannot be destroyed, for the agents by which such destruction could be accomplished would in nearly all cases decompose the poison ; it therefore becomes necessary to separate the alkaloid, in as pure a state as possible, from the foreign substances. In the operations necessary for this purpose, the extracts must be reduced to the smallest possible volume, the reagents used must be employed in small quantities, and the solutions used as tests must be applied by delicate glass rods ; for it must be remembered that the poison may be present in exceedingly small quantity, and that it is often impossible to separate more than a small proportion of that quantity in a sufficiently pure state to be tested.

We will only consider a few of the more commonly occurring alkaloids, first giving a general outline of the processes by which they may be separated from complex organic mixtures, and recognized by their characteristic reactions.

METHOD OF STAS.

§ 290. The method proposed by Stas for the separation of the alkaloids, depends upon the fact that the free alkaloids are generally soluble in ether, but not in water ; while their salts are soluble in aqueous liquids ; hence when an alkaloid is set free from one of its combinations dissolved in water, and the mixture is agitated with ether, the latter takes up the free alkaloid, and leaves it in a comparatively pure state on evaporation.

If the suspected substance be liquid, or if it consist of both solid and liquid, it is mixed with about twice its vol-

ume of strong alcohol containing from 0.5 to 2 grammes of tartaric acid, and the mixture is digested at about 70° , on a water-bath. If the matter be solid, such as the tissues of the stomach, liver, etc., it is cut into small pieces and digested at about 70° , with strong alcohol acidulated with tartaric acid as before; it is then pressed in a cloth, and the digestion with acid alcohol is repeated several times. The alcoholic solution is allowed to cool, filtered, and the residue on the filter is well washed with alcohol, the washings being added to the filtrate. The latter is then transferred to a retort, and evaporated at a temperature of about 35° , the distillation being hastened, if desired, by a current of air drawn through the liquid, by the aid of an aspirator. When the greater part of the alcohol has passed over, the operation is arrested, and the liquid is allowed to cool, and filtered through a filter which has previously been moistened with water. The acid filtrate is now agitated in a small flask or test-tube with several times its volume of ether; the latter is removed by decantation, as soon as it separates, and the operation is repeated with fresh ether as long as the latter continues to take up coloring matter. The ethereal solution may contain colchicine, digitaline, and foreign matters; the alkaloids remaining in the form of salts in the aqueous liquid, which is then mixed with powdered glass, to prevent the residue from becoming a solid mass, and evaporated to dryness in a vacuum or under a bell-jar over sulphuric acid. The residue is carefully triturated with absolute alcohol, and allowed to macerate for about twenty-four hours. The solution is then filtered, and the new filtrate is cautiously evaporated to dryness at a temperature not above 35° . The residue is dissolved in a very small quantity of water, potassium or sodium carbonate is added in sufficient quantity to set free the alkaloid, and the liquid is immediately agitated with four or five times its volume of pure ether. The ethereal layer is rapidly decanted, as soon as it separates, filtered into a watch-glass, and allowed to evaporate spontaneously. The residue in the watch-glass will contain the alkaloid, which may form oily streaks on the sides of the glass (conine, nicotine) or may be either an amorphous or a crystalline solid.

§ 291. The method of Stas answers very well for the separation of most of the alkaloids, and it has the undoubted advantage of leaving the poison in a nearly pure, and sometimes crystalline, condition. In the case of morphine, unless the manipulations with ether be executed with great rapidity, all of the alkaloid may separate in the crystalline state from the alkaline aqueous liquid, morphine being insoluble in ether, and no trace of it can then be found in the ethereal solution.

Various methods have been employed to obviate the difficulty occasioned by the exceedingly slight solubility of some of the alkaloids in ether. Rodger and Girdwood proposed to replace the latter solvent by chloroform, and this agent is especially well adapted for the separation of strychnine. Uslar and Erdmann preferred hot amylic alcohol, and, since morphine is quite soluble in this menstruum, while it is insoluble in ether, their process is applicable for the separation of that alkaloid; but amylic alcohol must be separated by the application of direct heat, and consequently the alkaloid is rarely deposited in a crystalline form.

GENERAL REAGENTS FOR THE DETECTION OF ALKALOIDS.

Various reagents have been proposed for the purpose of distinguishing the alkaloids from other bodies for which they might be mistaken. None of these are equally applicable to the whole class of compounds, and the nature of the body supposed to be an alkaloid must be decided by the totality of its properties. *Sodium phosphomolybdate*,—made by dissolving washed ammonium phosphomolybdate in sodium hydrate, evaporating to dryness, and calcining the residue, which is then dissolved in very dilute nitric acid,—precipitates all of the ordinary alkaloids. Mercurio-potassium iodide (mercuric chloride 13.546 gr., potassium iodide 49.8 gr., water one litre) is perhaps the most delicate general reagent for the alkaloids, forming with them precipitates which are usually crystalline. Circumstances will generally have pointed to the probable presence of a particular alkaloid, in which case these general tests will only be confirmatory.

CONINE.

§ 292. This alkaloid may be readily separated from organic mixtures by the process of Stas. After the evaporation of the ethereal solution of the alkaloid, oily drops or streaks will remain upon the watch-glass, and these will have the peculiar smell of conine, resembling that of hemlock; but this odor may in some cases be more or less marked by that of animal matters. Conine is soluble in about one hundred parts of water, and is freely soluble in alcohol, ether, chloroform, and benzol.

Chemical tests.—If a watch-glass containing a drop of hydrochloric acid be inverted over the glass containing the conine, the two glasses become filled with dense white fumes, and the conine is converted into a mass of crystalline needles of conine hydrochloride. If the conine be in aqueous solution, fumes appear in the glasses, conine hydrochloride is formed as before, and crystallizes from the solution when the latter is allowed to evaporate spontaneously. When conine is treated with strong aqueous hydrochloric acid, a red color is developed, and the liquid deposits crystals when sufficiently concentrated.

Solution of gold trichloride gives a bright-yellow, amorphous precipitate with solutions of conine or of its salts.

Iodine dissolved in aqueous potassium iodide, yields a reddish-brown, amorphous precipitate, which soon turns yellow, and redissolves in the liquid, unless an excess of iodine be present.

If chlorine water be added to an aqueous solution of conine, the mixture becomes turbid.

Solution of tannin forms a white, amorphous precipitate, soluble in a small quantity of hydrochloric acid, but reprecipitated on the addition of more acid.

Mercuric chloride gives, in aqueous solutions of conine, a white, curdy precipitate, which is only slightly soluble in water, but dissolves easily in mineral acids, and in acetic acid.

NICOTINE.

§ 293. Nicotine may be separated from organic mixtures by the process of Stas, but the operation is greatly facilitated by replacing the ether by chloroform, since nicotine is much more soluble in chloroform than in ether, under these circumstances.

Like conine, nicotine is left in oily drops or streaks after the evaporation of its solution in chloroform or ether; it has a pungent, suffocating odor, and an acid, burning taste. It is soluble in all proportions of water, thus differing from conine, and is also soluble in alcohol, ether, chloroform, and benzol.

Chemical tests.—If a watch-glass bearing a drop of hydrochloric acid be inverted over another glass containing a drop of nicotine, the glasses become filled with white fumes, but these fumes are not as dense as those produced by conine, and no crystals of nicotine hydrochloride are formed. In fact, the latter salt crystallizes with difficulty, and when pure nicotine is treated with aqueous hydrochloric acid, and the solution is allowed to evaporate, a syrupy liquid is obtained, without the formation of crystals.

Gold trichloride gives a yellow precipitate, somewhat darker in color than that produced with conine.

Iodine in potassium iodide produces an amorphous precipitate, varying from reddish-brown to yellow, according to the proportions of alkaloid and reagent present.

Chlorine water produces no turbidity in aqueous solutions of nicotine.

Tannin produces an abundant, white precipitate, which, when treated with hydrochloric acid, behaves like the precipitate produced by conine under the same circumstances.

Mercuric chloride throws down a white, curdy precipitate, which becomes yellow if much of the alkaloid be present, and at the same time groups of colorless crystals are deposited. If the solution of nicotine be dilute, the color of the precipitate does not change, but crystals

are eventually deposited, as in the case of stronger solutions.

These reactions are quite sufficient to distinguish nicotine from conine, the only alkaloid with which it is likely to be confounded in toxicological examinations.

MORPHINE.

§ 294. Opium and its principal alkaloid, morphine, are frequently employed criminally, and it sometimes becomes necessary to decide whether the latter alkaloid has been administered in a pure state as a salt, or whether poisoning has been caused by one of the preparations of opium. In the latter case, it is sufficient that meconic acid, with which morphine is naturally combined in opium, be detected, together with the alkaloid; then, if sufficient of the poison be recovered, it may be possible to prove the presence of one or more of the other alkaloids which exist in opium.

The separation of morphine from organic mixtures is a matter of great difficulty, and none of the processes so far proposed yield very satisfactory results, especially when the alkaloid is to be sought in blood or in the solid tissues of the economy. Sometimes Stas's method succeeds very well, but if the agitation with ether and subsequent separation of the liquids be not conducted with great rapidity; or if, as sometimes occurs, the ether separate slowly from the aqueous liquid, all of the morphine will be deposited in the crystalline state, and none can be detected after the evaporation of the ether. Nothing is gained by the use of chloroform, for morphine is almost insoluble in that liquid.

§ 295. To obviate this difficulty, Uslar and Erdmann suggested the use of hot amylic alcohol, in which morphine is quite soluble, and which separates readily from a watery liquid with which it has been agitated.

The suspected mixture is diluted with water, if necessary, and acidulated with hydrochloric acid; it is then di-

gested for several hours at a temperature of about 75° , on a water-bath, after which it is filtered through a cloth; the residue is again extracted with very dilute hydrochloric acid, and the extract, strongly pressed out, is added to the filtrate. The latter is then neutralized with ammonia, and evaporated to dryness on a water-bath. The residue is repeatedly extracted with hot amylic alcohol, and the solutions are united, and filtered through paper which has been previously moistened with amylic alcohol. The filtrate will contain fatty and coloring matters, together with morphine, should that alkaloid have been present in the original mixture. It is, therefore, agitated with three or four times its volume of hot water slightly acidulated with hydrochloric acid, and the alkaloid is thus converted into hydrochloride which is taken up by the water, while most of the other matters remain dissolved in the amylic alcohol. The latter is removed by the aid of a pipette, or a small separating funnel, and the aqueous liquid is repeatedly agitated with fresh portions of amylic alcohol, until most of its color is removed. It is then evaporated to a small volume, neutralized with ammonia, and agitated with hot amylic alcohol. The latter takes up the free alkaloid; after it has separated from the water it is carefully decanted, and the aqueous residue is again extracted with hot amylic alcohol. The alcoholic liquids are united, and evaporated to dryness on a water-bath. If the residue be dark-colored, it should be redissolved in very dilute hydrochloric acid, and the treatment with amylic alcohol repeated. However, it is often sufficiently pure for immediate examination, and, if amorphous, can be crystallized by dissolving it in a few drops of hot ordinary alcohol, and allowing the latter to evaporate spontaneously.

§ 296. Another process for the separation of morphine has been proposed by Dr. T. G. Wormley, and is well adapted for the detection of the alkaloid when it is suspected that opium or one of its preparations has been administered. It depends upon the fact that a mixture of alcohol and ether dissolves morphine, to a certain extent, and at the same time separates without difficulty from an aqueous liquid with which it has been agitated.

If the substance be a comparatively clear liquid, it is acidulated with acetic acid, evaporated to a small bulk on a water-bath, and filtered; if, however, the mixture be complex, the solids are divided as finely as possible, the mass is diluted, if necessary, with weak alcohol acidulated with acetic acid, and digested at a moderate temperature. The liquid is then strained through a cloth, and the residue is extracted with strong alcohol, and pressed, the washings being added to the filtrate. The latter is then evaporated to a small volume at a temperature of about 70° , and the residue is allowed to cool, and filtered through paper which has been moistened with water. An excess of lead acetate is added to the filtrate, and throws down any meconic acid that may be present, in the form of lead meconate; morphine and other opium alkaloids remain in solution. The precipitate is collected on a small, moistened filter, and washed with a little water, the washings being added to the filtrate.

a) The precipitate may contain lead meconate; it is transferred to a test-tube by piercing the bottom of the filter while still moist, and washing its contents into the tube by a stream of distilled water from a wash-bottle. The lead compound suspended in the water is then decomposed by gaseous hydrogen sulphide, and the lead sulphide thrown down is separated by filtration. Any meconic acid present will be found in the filtrate, which is to be concentrated to a small volume on a water-bath. The residue, which will have a brownish color, if it contain much meconic acid, is distributed in several watch-glasses, and examined by the tests indicated in § 298.

b) The filtrate is freed from the excess of lead acetate by gaseous hydrogen sulphide, and after the precipitate has subsided, the clear liquid is separated by filtration, and the filtrate evaporated to dryness on a water-bath. The residue is treated with a small quantity of water, and the liquid again filtered. The new filtrate is diluted, if necessary, rendered slightly alkaline by solution of potassium carbonate, and allowed to stand for a few moments. It is then agitated with several times its volume of anhydrous ether, to remove coloring matters

and opium principles other than morphine, and the ethereal solution is decanted and set aside for subsequent examination, if necessary.

The aqueous solution is now thoroughly agitated with four or five times its volume of a mixture of two parts of anhydrous ether with one part of pure alcohol; if the liquids do not separate readily after standing a few moments, their separation may be effected by the addition of a little pure ether. The layer of alcoholic-ether is decanted into a large watch-glass, and allowed to evaporate spontaneously, when the morphine will often be left in the crystalline form. The residue may be carefully washed by flowing a few drops of pure water over it, and will then be ready for chemical examination.

Chemical Tests.

MORPHINE.

§ 297. Concentrated nitric acid produces with morphine, or strong solution of its salts, a color varying from yellow to orange-red, according to the relative quantities of acid and alkaloid, and the dilution or concentration of the latter. The test succeeds best when a drop or two of the acid is placed upon the morphine or one of its salts in a dry state.

Iodic acid is reduced by morphine, iodine being set free; if, therefore, a strong solution of morphine or of one of its salts, or better, the alkaloid in the dry state, be treated with a drop or two of a concentrated solution of iodic acid, and a small quantity of starch paste be added, the characteristic blue color produced by free iodine and starch, will at once be developed. The reduction is also made evident by the addition of ammonia, which changes the yellowish color of the liquid to yellowish-brown. The iodic acid solution may be conveniently replaced by sodium iodate, to which an excess of sulphuric acid is added at the time of making the test.

Solution of ferric chloride produces a blue color in neutral solutions of morphine or its salts. The intensity of the color depends upon the quantity of ferric chloride, of which the solution employed should be very dilute,

and the reaction succeeds best with the alkaloid or its salt in a dry state. The blue color is destroyed by acids, alkalies and heat; nitric acid changes it to orange-red.

MECONIC ACID.

§ 298. When a solution of meconic acid is treated with a solution of a ferric salt, a blood-red color is developed, and this is unaffected by mercuric chloride or auric chloride, but is at once blackened by a solution of stannous chloride. This test is exceedingly delicate; a similar red color is produced by a ferric salt and an alkaline sulphocyanate, but this color disappears on the addition of mercuric chloride. Acetic acid also produces a red color with ferric salts, but acetic acid is not precipitated by lead acetate, as is meconic acid.

Lead acetate added to solutions of meconic acid, produces a yellowish precipitate of lead meconate, which is insoluble in acetic acid.

OPIUM ALKALOIDS OTHER THAN MORPHINE.

§ 299. Besides morphine, opium contains other alkaloids, of which the more important are codeine or methylmorphine, and narcotine. In the process of T. G. Wormley these are removed from the alkaline aqueous solution when it is agitated with ether to decolorize the liquid.

The most sensitive test for codeine is iodized potassium iodide; this reagent, having a pale yellow color, strikes a violet-red color with codeine. The latter alkaloid does not reduce iodic acid, nor does it produce any coloration with ferric chloride. Sulphuric acid gives a blue color.

Narcotine yields a red color when heated with concentrated sulphuric acid, and this changes to a violet when the dry residue is moistened with nitric acid. A little narcotine moistened with sulphuric acid, or a solution of narcotine treated with strong sulphuric acid, acquires a red color after standing for twenty-four hours, without the aid of heat.

STRYCHNINE.

§ 300. Strychnine is almost insoluble in ether; hence the method of Stas is not applicable to the separation of this alkaloid from organic mixtures. If, however, the ether be replaced by chloroform (Rodger and Girdwood's process), all of the alkaloid may be separated, for one part of strychnine only requires about eight parts of chloroform for its solution. Consequently, when the presence of strychnine is suspected, the aqueous solution which has been rendered acid by tartaric or acetic acid, is neutralized with potassium or sodium carbonate, and agitated with about an equal volume of pure chloroform; after the liquids have separated, the heavy chloroformic layer is removed, either by decanting off the upper layer or by the aid of a separating funnel, and is allowed to evaporate spontaneously. The residue may present crystals of strychnine; generally, however, in order to eliminate all foreign organic matter, the dry residue must be dissolved in a little distilled water containing a drop or two of acetic acid; the solution is filtered, neutralized with potassium or sodium hydrate, and again extracted by chloroform. The alkaloid is usually obtained in a crystalline form after the spontaneous evaporation of this second solution, and the chemical tests may be applied immediately.

CHEMICAL TESTS.

§ 301. Potassium and sodium hydrates and ammonia, produce in solutions of strychnine salts a white amorphous precipitate, consisting of the free alkaloid which soon assumes a crystalline form. Strychnine and all of its salts are peculiarly bitter, and the taste is used as confirmatory evidence to the chemical reactions.

When strychnine or one of its salts is treated with a little concentrated sulphuric acid, and potassium dichromate is added, a characteristic play of colors is manifested, a blue tint first appearing, which soon changes

to violet and red, and ultimately the color disappears altogether.

The test may be made in a porcelain capsule or in a watch-glass, and is best applied to the suspected substance in a solid state. The latter is moistened with a drop of concentrated sulphuric acid, in which the pure alkaloid will dissolve without change of color; a minute fragment of potassium dichromate is then placed in the drop of liquid, and moved around by the aid of a slender glass rod. If strychnine be present, a blue color is at once produced, and rapidly changes to violet and red,—then fades entirely. This test is exceedingly delicate and characteristic, but it may entirely fail if morphine be present. When, however, chloroform is used in the extraction of the alkaloid, or even when ether is used and the alkaline aqueous liquid is allowed to stand for some time before extracting it with ether, morphine cannot possibly be present. Sometimes the presence of certain organic substances which may be removed by chloroform from complex mixtures, interferes with this test. If a negative result be obtained, it is therefore advisable to add a trace of strychnine to a separate portion of the chloroform extract, and to test this in the usual manner; if this portion then respond to the color test, while no result can be obtained from the extract to which no strychnine was added, the absence of strychnine may be safely inferred.

Solution of potassium dichromate produces, in solutions of strychnine and its salts, a bright yellow crystalline precipitate of strychnine chromate. After removing the excess of liquid by careful decantation, or by means of a piece of filter-paper, this precipitate may be dried, and then moistened with a drop or two of concentrated sulphuric acid; a rich blue color will then be developed, changing to purple, violet, and red. By making the potassium dichromate test first, the color reaction may thus be afterwards tried upon the same portion of the suspected substance.

BRUCINE.

§ 302. Brucine is separated from organic mixtures by the same process which serves for the separation of strychnine. It is soluble in almost all proportions of chloroform, and is also freely soluble in alcohol.

The residue left after the evaporation of its solutions may be identified as brucine by the following tests:—

Brucine immediately assumes a blood-red color when moistened with a drop of concentrated nitric acid, and dissolves, forming a red solution. If this solution be heated, its color changes from red to orange or yellow; if now it be allowed to cool, and a drop of stannous chloride solution be added, a purple color is developed, but soon disappears if either the nitric acid or stannous chloride be employed in considerable excess.

Brucine dissolves in concentrated sulphuric acid, forming a rose-red solution; if a minute crystal of potassium dichromate be added to the mixture, an orange or brown color is produced, and gradually changes to green, chromic oxide being precipitated. Brucine may thus be readily distinguished from strychnine.

If a small crystal of potassium nitrate be added to the pale red solution of brucine in concentrated sulphuric acid, the liquid assumes an orange-red color.

PHYSIOLOGICAL TEST.

A determination of the physiological action of the substance, supposed to be an alkaloid, which may be extracted from the contents of the stomach, etc., is often of great value in the selection of the chemical tests. This is especially the case with strychnine and brucine. A very small quantity of the substance is required for the test, and the experiment may easily be made upon a frog or rabbit. Some poisons, such as digitaline, absolutely require that the physiological test shall be made for their certain identification.

DETECTION OF ALCOHOL IN ORGANIC MIXTURES.

§ 303. In cases where alcohol has been taken shortly before death, it may generally be detected in the contents of the stomach; the odor of the alcoholic preparation is often perceptible in such mixtures.

It is necessary, however, to separate the alcohol in a state of approximate purity. If the organic matter be viscous, it is diluted with water, but if quite fluid the dilution is unnecessary. It is exactly neutralized by potassium carbonate, should its reaction be acid, and is distilled on a water-bath, in a flask or retort connected with a good condensing apparatus. When about one-sixth of the liquid has passed over, the distillate is examined as follows:—

If it contain alcohol, it will usually have the peculiar odor of the alcoholic preparation from which it has been derived.

Its specific gravity will be less than that of water.

When mixed with dilute sulphuric acid and a few drops of potassium dichromate solution, the liquid becomes green, owing to the separation of chromic oxide; the odor of aldehyde may at the same time be observed. This reaction is not characteristic, but may serve to confirm other tests. If dilute alcohol be shaken up with an excess of solid and dry potassium carbonate in a test-tube, the greater part of the water will be taken up by the potassium carbonate, and two layers of liquid will be formed. The alcohol constitutes the upper layer, and will burn on the application of flame. Before agitating the dilute alcohol with potassium carbonate, it should be purified as much as possible by repeated rectification, only about one-half of the liquid being collected at each distillation.

By the aid of any good fractionating apparatus, small traces of alcohol may be separated from the urine without difficulty, after the ingestion of alcoholic liquids.

CHEMICAL EXAMINATION FOR THE DETECTION OF A POISON OF WHICH THE NATURE IS NOT KNOWN.

§ 304. As a rule, in toxicological examinations, some index is afforded to the nature of the poison present, before the chemical analysis is undertaken. Sometimes this index is furnished by a physical examination of the suspected substance; thus, if hydrocyanic acid, phosphorus, alcohol, chloroform, nitrobenzol, or other volatile poison be present, the characteristic odor of the matter generally guides the chemist to a certain extent in his selection of methods. The seeds of certain plants, stramonium, for example, or solid particles, such as arsenious oxide, may also indicate the probable poison to which particular attention must be directed.

Sometimes, however, none of these evidences are present, and the chemist is required to make an examination that shall include the whole range of poison that can be detected.

Volatile Poisons.

§ 305. The suspected mixture, rendered as homogeneous as possible by finely dividing the solid portions, is introduced into a large flask or retort, and distilled in a dark room. Phosphorus will show itself, if present, by a phosphorescent glow which fills the tube and the upper part of the condenser (§ 286). The condensed liquid is examined for hydrocyanic acid, as directed in § 284, and for alcohol, or any other volatile liquid, whose presence may be indicated by its peculiar odor.

The distillation must not be continued to dryness, nor must the temperature be allowed to rise above 100° , otherwise certain vegetable poisons might be destroyed.

Alkaloids.

§ 306. The contents of the retort are removed, and treated with alcohol and the required amount of tartaric

acid, and any alkaloids present are separated by the process of Stas (§ 290). However, after the final extraction of the alkaline aqueous solution with ether, the same operation should be repeated, the ether being replaced by chloroform (strychnine, brucine); and finally, after decanting the chloroform, the aqueous liquid should be agitated with hot amylic alcohol or with alcoholic ether (morphine).

The following compounds pass from the acid aqueous solution into the ether with which it is agitated to remove coloring matter (§ 290):—

Colchicine (partly). Digitaline. Picrotoxine.

The following alkaloids pass into the ether from the alkaline aqueous solution:—

Nicotine.	Conine.	Veratrine.
Narcotine.	Brucine (partly).	Strychnine (partly).
Aconitine.	Atropine.	Colchicine (partly).

The agitation with chloroform removes the remainder of any strychnine or brucine that may be present, while morphine remains in the alkaline liquid in which it was precipitated, and is extracted, as above indicated, either by alcoholic ether, or by hot amylic alcohol. The tests for these alkaloids are made upon the residues left after the evaporation of the ethereal, chloroformic, or amylic extracts; the properties by which the more commonly occurring alkaloids may be recognized, have already been described.

The aqueous residue left after the operations for the extraction of the alkaloids, may contain oxalic acid; a portion of it should therefore be tested as directed in § 280.

Metallic Poisons.

§ 307. The remainder of the aqueous mixture, together with the solid residue which was extracted by alcohol and tartaric acid, is mixed with hydrochloric acid, and may be directly tested by Reinsch's test for arsenic, antimony, and mercury. Whether the result be affirmative or negative, the mixture is heated on a water-bath, and powdered potassium chlorate is gradually

added until all of the solid matter is disintegrated and the liquid is fit for filtration. The liquid, which should be filtered hot, may contain the following metals:—

ARSENIC as arsenic acid.

ANTIMONY as antimony trichloride. If the presence of antimony be suspected, the digestion and treatment with potassium chlorate should be made in a retort, because antimony chloride may be lost by volatilization, if the mixture be heated in the open air. In this case, the liquid which distils from the retort must also be examined for antimony.

TIN as stannic chloride. The operation should be conducted in a retort, as in the case of antimony.

MERCURY as mercuric chloride.

LEAD as lead chloride: this may separate in crystalline plate from the filtrate as the latter cools.

COPPER as cupric chloride.

BISMUTH as trichloride; this forms a white precipitate on the addition of water, bismuth oxychloride being thrown down.

ZINC as chloride.

For the detection of any of these metals that may be present, the filtrate is saturated with hydrogen sulphide.

Yellow precipitates are formed by arsenic, tin, and cadmium.

An orange-colored precipitate by antimony.

Brownish-black by bismuth.

Black by mercury, silver, copper, and lead. If the liquid be not completely saturated with hydrogen sulphide, lead will be precipitated as sulpho-chloride, which is red or brownish.

After filtration from any precipitate formed by the hydrogen-sulphide, the liquid is partially neutralized with ammonia; this is best accomplished by removing a small portion of the liquid, completely neutralizing the remainder, and then again mixing the two portions. Sufficient sodium acetate is then added to replace all of the free hydrochloric acid by acetic acid, and the liquid is again saturated with hydrogen sulphide. Any zinc present will be precipitated as zinc sulphide.

These metallic sulphides are subsequently treated as has

been directed, for the identification of the different metals individually.

The residue left on the filter may contain silver or lead in the form of chloride. It is dried, strongly calcined with sodium carbonate and sodium nitrate, and the fused mass is dissolved in water, and examined for small globules of metal.

The liquid from which zinc may or may not have been precipitated by hydrogen sulphide, is boiled to expel hydrogen sulphide, rendered strongly acid with hydrochloric acid, and sulphuric acid is added. A white precipitate indicates the presence of barium; this precipitate is collected, and the presence of barium confirmed by appropriate tests.

APPENDIX.

VOLUMETRIC ANALYSIS.

1. THE principles of volumetric analysis, of which numerous examples have been given in this work, are of almost universal application.

The solutions which are usually employed in the analysis of urine and in other clinical analyses, are *standard* solutions, so graduated that the calculation of the results may be as little complicated as possible. They are usually made so that each cubic centimetre shall represent 5 or 10 milligrammes of the substance to be estimated.

In general analysis, *normal* and *decinormal* solutions are employed, these names being applied to solutions which are volume for volume equivalent to each other. Such solutions may evidently be made by dissolving equivalent proportions of the respective substances in one litre of water; for instance, a litre of normal hydrochloric acid, containing 36.5 grammes of the acid, and a litre of normal sodium hydrate, containing 40 grammes of the latter compound, would reciprocally neutralize each other, 36.5 being the molecular weight of hydrochloric acid and 40 being the molecular weight of sodium hydrate, and these two substances reacting upon each other molecule for molecule. Then, of compounds such as sodium hydrate and hydrochloric acid, normal solutions are made by dissolving in water a quantity of the compound equal to its molecular weight expressed in grammes, and diluting the liquid to exactly one litre.

One molecule of sulphuric acid will react with and neutralize two molecules of sodium hydrate, hence one litre of normal sulphuric acid, which must be equivalent

to one litre of normal sodium hydrate, must contain, not 98 grammes of sulphuric acid, 98 being the molecular weight of H_2SO_4 , but 49 grammes, or half the molecular weight expressed in grammes.

Decinormal solutions are of exactly one-tenth the force of normal solutions, and are usually made by diluting 100 c. c. of the corresponding normal solution to one litre.

When one normal solution has been prepared of exact strength, the others may readily be made. A convenient solution to prepare first is that of sodium carbonate; the molecular weight of this substance, perfectly pure and dry, is 106; since its molecule contains two atoms of sodium, 53 grammes of pure sodium carbonate are dissolved in water, and the solution is diluted to one litre. Each cubic centimetre of this solution contains 53 milligrammes of the salt.

Normal sulphuric acid may then be made by diluting 49 grammes of pure concentrated sulphuric acid to about 950 cubic centimetres. This solution is not exact, and must be titrated with the normal sodium carbonate. A burette is filled to the zero line with the normal acid, and a convenient quantity, say 20 c. c., of the sodium carbonate solution is measured into a beaker, and enough litmus solution is added to communicate a pale-blue color to the liquid. It is then necessary to determine how much of the acid solution is required to neutralize the sodium carbonate, as indicated by the change of color of the liquid; if the acid were exactly normal, 20 c. c. should be required. Since carbonic acid is set free, and is in part absorbed by the liquid, the litmus would be reddened too soon if the carbon dioxide were not expelled, for carbonic acid itself will redden litmus. Therefore the liquid is heated to boiling before adding the acid from the burette, and the sulphuric acid is then allowed to flow in, with constant stirring, until the color of the liquid changes. The solution is again boiled, and the blue color will probably return; more acid is then added until the red color becomes permanent. It must be remembered that it is a *change* of color, and not an

intensity of tint, that indicates the termination of the experiment.

The quantity of the sulphuric acid used must have contained 980 milligrammes of sulphuric acid, for it has exactly neutralized 20 c.c. of a solution of sodium carbonate, of which each cubic centimetre contained 53 milligrammes. As the acid was somewhat stronger than required, less than 20 c.c. will have been employed; suppose 19.2 c.c. were actually used; then for every 19.2 c.c., 0.8 c.c. of water should be added. The exact volume of the dilute acid remaining is therefore ascertained, and 0.8 c.c. of water are added for every 19.2 c.c. of solution. The acid, which should now be exactly normal, is again titrated with normal sodium carbonate as before, in order that its accuracy may be verified.

With these two normal solutions, all other normal solutions of alkaline or acid compounds may be prepared. For instance, 40 grammes of sodium hydrate are dissolved in 900 c.c. of water, and the solution is titrated with the normal sulphuric acid, and diluted so that the two liquids shall be equivalent, volume for volume. It is not necessary to heat the liquid in this case, nor in any other acidimetric or alkalimetric titration in which no carbonic acid is liberated.

Preparation of Normal Solutions.

NORMAL SODIUM CARBONATE. 1 LITRE = 53 GRAMMES
 Na_2CO_3 .

2. It is not always easy to procure chemically pure sodium carbonate, but pure sodium acid-carbonate may always be obtained, and is indeed preferable for the preparation of this solution. 85 grammes of sodium acid carbonate are heated to incipient redness in a platinum or porcelain dish, and when the salt is entirely reduced to the neutral carbonate, which will require ten or fifteen minutes, it is allowed to cool in a desiccator. When cold, the residue is rapidly weighed, the small quantity in excess of 53 grammes is removed, and the residue dissolved in distilled water. The solution, with the washings of the dish in which the carbonate was heated

and of that in which the solution was effected, is introduced into a litre flask, and diluted to exactly one litre with distilled water.

NORMAL SULPHURIC ACID. 1 LITRE = 49 GRAMMES
 H^2SO^4 .

3. The preparation of this solution has already been described; it must be titrated with the normal sodium carbonate, and diluted to exactly the required strength.

NORMAL NITRIC ACID. 1 LITRE = 63 GRAMMES HNO^3 .

4. Normal nitric acid must be used instead of normal sulphuric acid when calcium, strontium, or barium hydrates or carbonates, are to be dissolved, since the sulphates of these metals are insoluble. The solution is made by diluting about 50 cubic centimetres of pure concentrated nitric acid, with distilled water, to about 950 c.c., and titrating the liquid with normal sodium carbonate and correcting it, precisely as has been directed for the preparation of the normal sulphuric acid.

NORMAL SODIUM HYDRATE. 1 LITRE = 40 GRAMMES
 NaOH .

5. The sodium hydrate employed must be quite pure, and free from carbonate. As it cannot be obtained absolutely dry, it is necessary to dissolve rather more than the required quantity in order to make one litre of the solution; 42 or 43 grammes are dissolved in 800 or 900 c.c. of water, and when cold the solution is titrated with the normal sulphuric acid, as already indicated, and diluted as found necessary. This solution must be preserved in well-stoppered bottles, that it may not absorb carbon dioxide from the air.

6. With these acid and alkaline normal solutions and the corresponding decinormal solutions, it is only possible to estimate directly the amount of free acid, alkali or alkaline carbonate in any given liquid, the nature of the

substance to be estimated of course being known; but by indirect processes, these same solutions may serve for the estimation of a great number of substances, and are of almost unlimited application.

The estimation of the acidity of the urine by means of decinormal sodium hydrate, has already been explained (§ 128).

The examples which follow are such as require but little apparatus, and will serve to illustrate the application of acidimetry and alkalimetry.

ESTIMATION OF CARBONIC ACID GAS, FREE AND COMBINED, IN WATER.

7. This method depends upon the precipitation of barium carbonate by the action of carbonic acid on baryta water, and upon the decomposition of alkaline carbonates when boiled with baryta water, the same precipitation taking place. The exact strength of the baryta water is first found by titration with decinormal nitric acid, and the solution must then be preserved out of contact with the air.

A measured volume of the water (200–500 c.c.) is boiled with a slight excess of the baryta-water, exactly measured, in a flask, and the latter is corked and allowed to cool. About half of the clear liquid is then decanted or transferred by the aid of a pipette into a beaker, and the excess of barium hydrate is exactly titrated with decinormal nitric acid. As the decomposition of any alkaline carbonate present will have introduced into the solution a quantity of alkaline hydrate exactly equivalent to that of the barium hydrate which effects such decomposition, the difference between the volume of decinormal acid required and that which would have been used had the alkalinity of the barium solution not been disturbed, will correspond to the free carbonic acid present, together with half of that which existed in combination as acid carbonates.

The precipitate in the flask is then collected on a filter, thoroughly washed with boiling water, and filter and precipitate together are placed in a beaker and treated with an exactly measured volume of decinormal nitric

acid. The excess of the latter is then estimated by decinormal sodium hydrate; the difference indicates the total amount of carbonic acid present in the water, all having been precipitated as barium carbonate.

Example.—250 c.c. of mineral water are boiled with 50 c.c. of baryta-water, which has been found by previous trituration to be exactly equivalent to 56 c.c. of decinormal nitric acid. After cooling, 150 c.c. of the liquid (the total volume after mixing was 300 c.c.) are found to require 9.3 c.c. of decinormal nitric acid for perfect neutralization, as indicated by either litmus solution or turmeric paper; the 300 c.c. would therefore have required 18.6 c.c.; and the difference between this and 56 c.c. = 37.4 c.c., expresses in nitric acid the quantity of free carbonic acid in the water, and half of that which existed as acid carbonates. But each cubic centimetre of decinormal nitric acid contains 6.3 milligrammes of nitric acid, and this is equivalent to 2.2 milligrammes of carbonic acid gas; therefore $37.4 \times 2.2 = 82.28$ milligrammes of carbon dioxide were contained, free and as acid carbonates, in the 250 c.c. of water analyzed.

The precipitate is collected, washed, and dissolved in 50 c.c. of decinormal nitric acid, and the excess of acid is estimated by decinormal sodium hydrate, of which 6.1 c.c. are required. Since the acid and alkali are equivalent to each other, volume for volume, 43.9 c.c. of the nitric acid were neutralized in dissolving the barium carbonate, and correspond to an equivalent quantity of carbon dioxide which was disengaged. Therefore the total carbonic acid of the 250 c.c. of water = $2.2 \times 43.9 = 96.58$ milligrammes. The difference $96.58 - 82.28 = 14.30$ milligrammes may be formulated as that which exists in combination; but as solutions containing free carbonic acid do not contain carbonates, but acid carbonates, containing double the proportion of acid contained in the neutral salts, it is more correct to divide the carbon dioxide between acid carbonates and free carbonic acid. Thus—

As acid carbonate	0.0286
As free acid	0.0680
Total CO ²	<hr/> 0.0966

ESTIMATION OF AMMONIA.

8. In solutions of ammoniacal salts, the ammonia may be estimated by a very simple process, provided the solution be neutral. If it be acid or alkaline, it must be exactly neutralized. It is then boiled with an excess of normal or decinormal sodium hydrate, until all of the ammonia is expelled, and the excess of alkali is estimated by normal nitric acid.

Example.—100 c.c. of an ammoniacal liquid are exactly neutralized, and boiled with 20 c.c. of normal sodium hydrate until the vapors disengaged no longer have any action on red litmus paper. The liquid is then titrated with normal sulphuric acid, of which it is found that just 13.7 c.c. are required to effect neutralization; 6.3 c.c. of sodium hydrate have therefore been used in decomposing the ammoniacal salt; but each cubic centimetre contained 40 milligrammes of sodium hydrate, which would expel 17 milligrammes of ammonia. The 100 c.c. of liquid analyzed therefore contained $17 \times 6.3 = 0.1071$ gr. of ammonia.

ESTIMATION OF COMBINED ACID IN NEUTRAL SALTS.

9. This method is only applicable when the salt is entirely decomposed by an excess of an alkaline hydrate or carbonate, a metallic hydrate or carbonate being precipitated.

A weighed quantity of the salt, or a measured volume of its solution, should it be in solution, is treated with an excess of normal sodium hydrate or sodium carbonate, as may be required; the mixture is then diluted to a known volume, and the excess of alkali is estimated in the clear liquid after the precipitate has subsided.

Example.—It is required to estimate the proportion of chlorine in a solution of barium chloride, or in the dry salt. In the latter case, one or two grammes of the salt are dissolved in water, 20 c.c. of normal sodium carbonate are added, and the liquid is diluted to say 200 c.c.; if the salt be in solution, 50 or 100 c.c. are treated with 20 c.c. of normal sodium carbonate, and the whole is

diluted to 200 c.c. as before. After the precipitate has subsided, 100 c.c. of the clear liquid are removed, placed in a beaker, and titrated with normal nitric acid. Suppose 2.1 c.c. of the latter be required: then, since only half of the liquid is operated upon, 4.2 c.c. would be required by the 200 c.c., each of which has neutralized one cubic centimetre of sodium carbonate. Therefore $20 - 4.2 = 15.8$ c.c. of normal sodium carbonate were used in precipitating the barium salt, and, since each c.c. contains 40 milligrammes of sodium hydrate, it corresponds to 35.5 milligrammes of chlorine. Then the quantity of BaCl^2 under analysis contained $35.5 \times 15.8 = 0.4609$ gr. of chlorine.

Of course the acid of any other soluble barium salt could be estimated in the same manner, and the method is capable of very extended application.

10. The estimation of chlorine in urine by means of a standard solution of silver nitrate is described in § 153. In general analysis, a normal and a decinormal solution of silver nitrate, containing respectively 170 grammes and 17 grammes of the salt per litre, are used instead of the standard solution.

11. The estimation of iron volumetrically by means of potassium permanganate, has been given in section 182, on the estimation of hemoglobin in blood.

STANDARD SOLUTIONS.

12. For the sake of convenience in the calculation of analyses, the solutions employed in the volumetric analysis of urine are so made that each cubic centimetre may represent either five or ten milligrammes of the substance to be estimated: the volumes of urine analyzed are then so selected that the number of cubic centimetres of standard solution used, represents either the number of grammes of the substance contained in one litre of urine, or a decimal fraction of that quantity.

Thus 1 c.c. of the solution of mercuric nitrate, used

for the estimation of urea (§ 147), corresponds to 0.010 gr. of urea. In this method 10 c. c. of urine are analyzed; should these require 10 c. c. of mercury solution, one litre of urine would evidently contain 10 gr. of urea. The number of cubic centimetres used, therefore, corresponds to the number of grammes of urea in one litre of urine.

In the preparation of the mercuric nitrate solution, of which one litre must precipitate 10 grammes of urea as a compound of one molecule of urea nitrate with two molecules of mercuric oxide, somewhat more than the calculated quantity of mercuric oxide must be used in order to produce the final reaction with the sodium carbonate. The quantity required by the formula $\text{CON}^2\text{H}^4 + 2\text{HgO}$, would be $\frac{432 \times 10}{60} = 72$ gr. per

litre, 60 being the molecular weight of urea, and 216 that of mercuric oxide. It is found that a sufficient excess of mercuric oxide is present if 77.2 gr. (corresponding to 96.855 gr. of mercuric chloride, in which form the mercury is best weighed), be employed. One litre therefore contains 5.2 gr. of mercuric oxide in excess of that absolutely required for 10 gr. of urea. It has been mentioned (page 109) that this estimation is only exact when 100 c. c. of the liquid analyzed contain two grammes of urea: the reason is obvious, for the solution is standardized by a two per cent. solution of urea; 15 c. c. of the latter would require 30 c. c. of mercuric nitrate, containing $5.2 \times 30 = 156$ milligrammes of mercuric oxide in excess, which would exist in 45 c. c. of liquid. Each c. c. would therefore contain 3.47 milligrammes of mercuric oxide to react with the sodium carbonate. Suppose the 15 c. c. to contain twice as much urea as before: then 60 c. c. of mercury solution would be required, and 75 c. c. of the mixture would contain $60 \times 5.2 = 312$ milligrammes of mercuric oxide to react with the sodium carbonate, that is, each c. c. would contain 4.16 milligrammes, or 0.69 milli. more than in the former case. It is evident that the reaction with sodium carbonate would then take place too soon, and the liquid would contain more

urea than indicated by the analysis, unless it were properly diluted.

In the method for the estimation of sodium chloride (§ 153), exactly the quantity of fused silver nitrate required to react with 10 grammes of sodium chloride, is dissolved in one litre of water,

$$58.5 : 10 :: 170 : x,$$

58.5 being the molecular weight of sodium chloride, and 170 that of silver nitrate. The number of cubic centimetres of the silver solution required by the residue of 10 c. c. of urine, represents the number of grammes of sodium chloride in one litre of urine.

The uranium solution for the estimation of phosphoric acid (§ 154), only gives accurate results when the analyses are made under the same conditions as the titration of the standard solution. Since 50 c.c. of urine are analyzed, the force of the uranium solution (1 c.c. = 0.005 gr. P^2O_5) has the convenience that one-tenth the number of centimetres used expresses the number of grammes of phosphoric anhydride in one litre of urine. Thus if 18 c.c. of the uranium solution be required to precipitate 50 c.c. of urine, one litre of the latter will contain 1.8 gr. of phosphoric anhydride.

Weights and Measures.

	Grains.	Ounces Troy = 480 grains.	Pounds Avoirdupois.
1 Milligramme =	0.01543	0.000032	0.0000022
1 Centigramme =	0.15432	0.000321	0.0000220
1 Decigramme =	1.54323	0.003215	0.0002204
1 Gramme =	15.43234	0.032150	0.0022046
1 Decagramme =	154.32349	0.321507	0.0220462
1 Hectogramme =	1543.23488	3.215072	0.2204621
1 Kilogramme =	15432.34880	32.150726	2.2046212

1 Grain = 0.064799 grammes.

1 Oz. Troy = 31.103496 grammes.

1 lb. avoirdupois = 0.453495 kilogrammes.

1 oz. avoirdupois = 28.343437 grammes.

To convert centigrade degrees into Fahrenheit degrees, multiply by 9, divide the product by 5, and add 32° .

To convert Fahrenheit degrees into centigrade degrees, subtract 32° , then multiply by 5 and divide by 9.

1 Metre = 39.370708 inches.

1 Centimetre = 0.393707 "

1 Millimetre = 0.039370 "

1 inch = 2.539954 centimetres.

INDEX.

- A** CID, acetic, 18
benzoic, 25
butyric, 18
capric, 18
caproic, 18
caprylic, 18
cholic, 58
formic, 17
glycocholic, 56
hippuric, 37
 estimation in urine, 115
hydraerylic, 21
hydrochloric, detection, in
 cases of poisoning, 265
 estimation, in gastric juice,
 191
hydrocyanic, detection in
 cases of poisoning, 269
 detection in solution, 271
 in vapor, 269
 estimation, 272
 tests for, 270, 271
lactic, 20
meconic, detection in cases of
 poisoning, 282, 284
nitric, detection in cases of
 poisoning, 266
oxalic, 22
 detection in cases of poison-
 ing, 268
 estimation, 269
oxaluric, 45
paralactic, 21
phosphoglyceric, 59
propionic, 18
succinic, 24
 detection in urine, 25
sulphuric, detection in cases
 of poisoning, 264
 estimation in ashes, 224
 in urine, 121
taurocholic, 57
uric, 39
- Acid, uric—
 estimation, 114
 valeric, 18
Acids, biliary, 56-58
 detection in blood, 145
 in urine, 97
 Pettenkofer's test for, 56, 97
fatty, 17
 detection and separation, 19
mineral, detection in cases of
 poisoning, 262
 detection in stains on cloth-
 ing, 267
 estimation in neutral salts,
 299
- Albumen, 65
 detection in urine, 94
 estimation, 66
 estimation in blood, 122
 in serous liquids, 168
 in urine, 122
- Albumen of white of egg, 66
- Albuminoid bodies, 61
 classification of, 64
 detection of, 63
 general properties, 62
- Albuminose, 73
- Alcohol, detection in complex
mixtures, 288
- Alkaloids, detection in cases of
poisoning, 275
 general reagents for, 277
 physiological test for, 287
 separation from organic mix-
 tures, 275
- Ammonia, detection in blood,
145
 in urine, 93
 estimation in urine, 125
 volumetric estimation of, 299
- Ammonium urate calculi, 136
sediment, 133
- Analysis, volumetric, 293

Animal ferments, 74
 pigments, 78
 Antimony, detection in cases of
 poisoning, 244
 quantitative estimation, 247
 separation from arsenic, 246
 tests for, 244
 Arsenic, detection in cases of
 poisoning, 229
 electrolytic test, 241
 Marsh's test, 237
 ordinary compounds of, 229
 quantitative estimation, 243
 Reinsch's test, 236
 separation from organic mix-
 tures, 235
 trisulphide, 229
 Arsenical pigments in paper-
 hangings, 235
 Arsenious oxide, 230
 liquid tests for, 232
 reduction test, 231
 sublimation test, 230
 Ashes of animal substances, 219
 estimation of calcium, 224
 of chlorine, 225
 of iron phosphate, 223
 of phosphoric acid, 225
 of potassium and sodium,
 223
 of sulphuric acid, 224
 qualitative analysis of, 219
 quantitative analysis of, 223

BARIUM, detection in cases of
 poisoning, 292
 Bile, general properties of, 193
 Biliary calculi, 194
 pigments, 79
 detection in urine, 98
 in blood, 145
 Gmelin's test for, 80
 Bilifuscin, 81
 Biliprasin, 81
 Bilirubin, 79
 Biliverdin, 80
 Bismuth, detection in cases of
 poisoning, 291
 Blood, 140
 abnormal constituents, 140
 analysis of, 143
 anatomy of, 140, 158

Blood—
 calculation of analyses, 148,
 152
 corpuscles, 159
 detection of ammonia in, 145
 of biliary matter, 145
 of carbon monoxide, 146
 of creatine and creatinine,
 144
 of glucose, 144
 of mineral salts, 144
 estimation of albumen, 148,
 152, 154
 of cholesterin, 155
 of fibrin, 150
 of hemoglobin, 157
 of lecithine, 156
 of mineral salts, 149
 of solid matter, 149
 of water, 149
 general chemical properties,
 141
 composition, 147
 in urine, 102
 normal constituents, 140
 quantitative analysis, 146
 serum of, 141
 analysis, 153
 Blood stains, detection of, 161
 Blue pus, 173
 Bone, 180
 analysis of, 181
 calculation of analysis, 184
 composition of, 186
 estimation of calcium, 182
 of carbon dioxide, 181
 of magnesium, 183
 of organic matter, 183
 of phosphoric acid, 183
 general chemical properties,
 180
 Brucine, detection in cases of
 poisoning, 287
 Butter, estimation in milk, 200
 rapid approximate estimation,
 204

CALCIUM, estimation in ashes,
 224
 in bone, 182
 Calcium oxalate, 23
 as urinary sediment, 133

Calcium oxalate—
 as urinary calculi, 138
 Carbon dioxide, estimation in
 bone, 181
 in water, 297
 Carnine, 44
 Casein, 74
 estimation, 199
 in milk, 198
 Chlorine, estimation in ashes,
 225
 in urine, 117
 Cholesterin, 32
 estimation in biliary calculi,
 195
 in blood, 155
 Choline, 58
 Chondrin, 77
 Codeine, detection in cases of
 poisoning, 284
 Colostrum, 212
 analysis of, 214
 Conine, detection in cases of
 poisoning, 278
 Copper, detection in cases of
 poisoning, 257
 occurrence in biliary calculi,
 195
 quantitative estimation, 260
 tests for, 259
 Cream, composition of, 196
 proportion in milk, 198
 Creatine, 46
 detection in blood, 144
 in muscular juices, 175
 Creatinine, 48
 detection in urine, 49
 estimation in urine, 116
 Cystine, 59
 detection in urine, 100
 in urinary calculi, 137

DESICCATION, 15 Dialysis, 14

EGG albumen, 66
 Evaporation, 14
 Excrements, 217
 Excretin, 33
 Expecterations after thoracentesis,
 171

Extraction, 14
 Extracts of glands, 179
 of muscles, 175

FATTY acids, 17
 Ferments, animal, 74
 Fibrin, 71
 detection in serous liquids, 167
 estimation in blood, 150
 Fibrinogen and fibrinoplasmin,
 71
 Filtration, 14
 Formic acid, 18

GASTRIC juice, 190
 analysis of, 191
 estimation of hydrochloric
 acid, 191
 Gelatin, 77
 Glandular juices, 179
 Globulin, 67
 Glucose, 27
 Böttger's test, 28
 detection in blood, 144
 in urine, 95
 estimation in urine, 123
 Fehling's test, 28
 Moore's test, 27
 Trommer's test, 27
 Glycogen, 31
 Gmelin's test for biliary pig-
 ments, 80
 Guanine, 44

HEMATIN, 70
 detection in blood stains,
 163
 hydrochloride, 70
 Hemin crystals, 70
 obtained from blood stains,
 164
 Hemoglobin, 68
 detection in blood stains, 163
 in urine, 102
 estimation in blood, 157
 reduced, 69
 Hippuric acid, 37
 estimation in urine, 115
 Hydrobilirubin, 81
 Hydrocele, effusion in, 170

Hydrocyanic acid, 269

Hydropisin, 67

Hypoxanthine, 43

extraction from muscular
juices, 177

INCINERATION, 16

Indican, 83

detection in urine, 84

Indigotine, 84

Indirubin, 85

Inosite, 29

detection in urine, 133

extraction from muscles, 177

LACTIC acid, 20

detection in urine, 97

Lactobutyrometer, 204

Lactodensimeter, 209

Lactose, 30

estimation in milk, 197, 204

Lead, detection in cases of poi-

soning, 253

detection in water, 256

in organic mixtures, 253

quantitative estimation, 256

tests for, 255

Lecithine, 58

Leucine, 50

detection in urine, 100

MARSH'S test, 237

Meconic acid, detection, 282,
284

Melanine, 78

Mercury, detection in cases of

poisoning, 248

electrolytic test, 251

quantitative estimation, 252

separation from organic mix-
tures, 249

tests for, 249, 250

Metalbumen, 68

Milk, analysis of, 197

Baumhauer's method of analy-
sis, 202

casein in, 198

Chevalier and Henry's method
of analysis, 201

Milk—

commercial analysis of, 209

cow's, 207, 208

density of, 197, 209

detection of adulterations,
209

diseased, 211

estimation of butter, 200, 204

of casein, 199, 206

of fat, 199

of lactose, 199, 204

of mineral salts, 200

ewe's, 208

general properties, 196

goat's, 208

Lehmann's method of analy-
sis, 206

mare's, 208

Millon and Commaille's me-
thod of analysis, 199

proportion of butter, 206

of cream, 198

of mineral salts, 207

of water, 197

rapid estimation of butter by
lacto-butyrometer, 204

sow's, 208

various analyses of, 207, 208

woman's, 208

Millon's reagent, 62

Moore's test for glucose, 27

Morphine, chemical tests for, 283
detection in cases of poisoning,
280

Uslar and Erdmann's method,
280

Wormley's method, 281

Mucin, 78

Muscular juices, 175

analysis of, 175, 177

Myosin, 72

NARCOTINE, detection in cases
of poisoning, 284

Neurine, 53

Nicotine, detection in cases of
poisoning, 279

Nitric acid, detection in cases of
poisoning, 266

normal solution of, 296

Normal solutions, 293

- OPIUM**, detection in cases of poisoning, 280
- Ossein, 77
- Oxalic acid, 22
detection in cases of poisoning, 268
estimation, 269
- Oxaluric acid, extraction from urine, 45
- Oxyhemoglobin, 68
- PANCREATIC** ferments, 76
- Pancreatin, 67
- Paralbumen, 67, 168
- Pepsin, 75
- Peptones, 64, 73
- Pettenkofer's test for biliary acids, 56, 97
- Phosphoric acid, detection in ashes, 220, 222
estimation in-ashes, 225
in bone, 183
in urine, 118
- Phosphorus, detection in cases of poisoning, 273
- Pigments, animal, 78
- Pleuritis, effusion in, 170
- Poisons, detection of, 227
alkaloid, 275, 289
mineral, 290
volatile, 289
- Ptyalin, 74
detection in saliva, 188
- Pus, 172
blue, 173
- Pus corpuscles, 173
- Pyin, 172
- Pyocyanin, 173
- Serum of blood, 141
analysis of, 153
- Sodium carbonate, normal solution of, 296
- Sodium hydrate, normal solution of, 296
- Solution, 13
- Spermatic fluid and stains, 214
- Spermatozooids, 215
in urine, 130
- Stains, blood, detection of, 161
mineral acids, detection of, 267
seminal, detection of, 214
- Standard solutions, advantages of, in analysis of urine, 300
preparation of, 300
- Stercorin, 33
- Strychnine, detection in cases of poisoning, 285
- Sulphate of indigo, detection in cases of poisoning, 264
- Sulphuric acid, detection in cases of poisoning, 264
estimation in ashes, 224
in urine, 121
normal solution of, 296
- Syntonin, 73
- TARTAR** emetic, detection in cases of poisoning, 244
- Taurine, 54
- Tin, detection in cases of poisoning, 247
- Tyrosine, 52
detection in urine, 100
- UREA**, 34
detection, 37
detection in blood, 143
estimation in urine, 106
Esbach's method, 113
Liebig's method, 106, 301
Yvon's method, 110
extraction from urine, 35
- Urea nitrate, 35
- Urea oxalate, 36
- Ureometer, 111
- Uric acid, 39
as urinary sediment, 127, 133
- REINSCH'S** test, 236
- SALIVA**, 187
analysis of, 188
- Salivary calculi, 189
- Sarcine, 43
- Scheele's green, 229
- Serolin, 33
- Serous effusions, special, 170
- Serous liquids, analysis of, 167
quantitative analysis of, 169

Uric acid—

- detection, 40
- detection in blood, 143
- in muscles, 177
- estimation in urine, 114
- extraction from small quantities of liquid, 41
- murexide test for, 40

Uric acid calculi, 136

Urinary calculi, 134

- albuminoid, 137
- ammonium urate, 136
- analysis of, 135
- calcium oxalate, 138
- cystic, 137
- mixed, 135, 139
- mulberry, 138
- phosphatic, 138
- uric acid, 136
- xanthic, 137

Urinary sediments, 125

- organized, 130
- unorganized, 127

Urine, 87

- abnormal constituents, 89
- accidental constituents, 89
- alkaline, 93
- approximate composition, 104
- blue and violet, 100
- cause of acidity, 92
- chemical examination, 90
- color, 94
- consistence, 90
- detection of abnormal coloring matter, 99
 - of albumen, 94
 - of ammonia, 103
 - of biliary acids, 97
 - of biliary pigments, 98
 - of blood, 102
 - of cystine, 100
 - of fat, 133
 - of glucose, 95
 - of inosite, 96
 - of lactic acid, 97
 - of leucine, 101
 - of tyrosine, 101
- estimation of albumen, 122

Urine, estimation—

- of ammonia, 125
- of creatinine, 116
- of glucose, 123
- of hippuric acid, 115
- of mean daily acidity, 92
- of mineral salts, 105
- of phosphoric acid, 118
- of sodium chloride, 117
- of solid constituents, 105
- of sulphuric acid, 121
- of urea, 106
- of uric acid, 114
- of water, 105
- normal constituents, 88
- physical properties, 87
- quantitative analysis, 103
- quantity, 90
- rapid qualitative analysis, 103
- reaction, 91
- red, hepatic, 98
- specific gravity, 91

Urobilin, 82

Urochrome, 83

Urocyanin, 85

Uroglauzin, 84

Urohematin, 83

Uroxanthin, 83

Urrhodine, 85

VITELLIN, 67

WEIGHTS and measures, 303

XANTHIN, 42

- extraction from muscular juices, 177

ZINC, detection in organic mixtures, 261

Zinc lactate, 21

Zinc paralactate, 22

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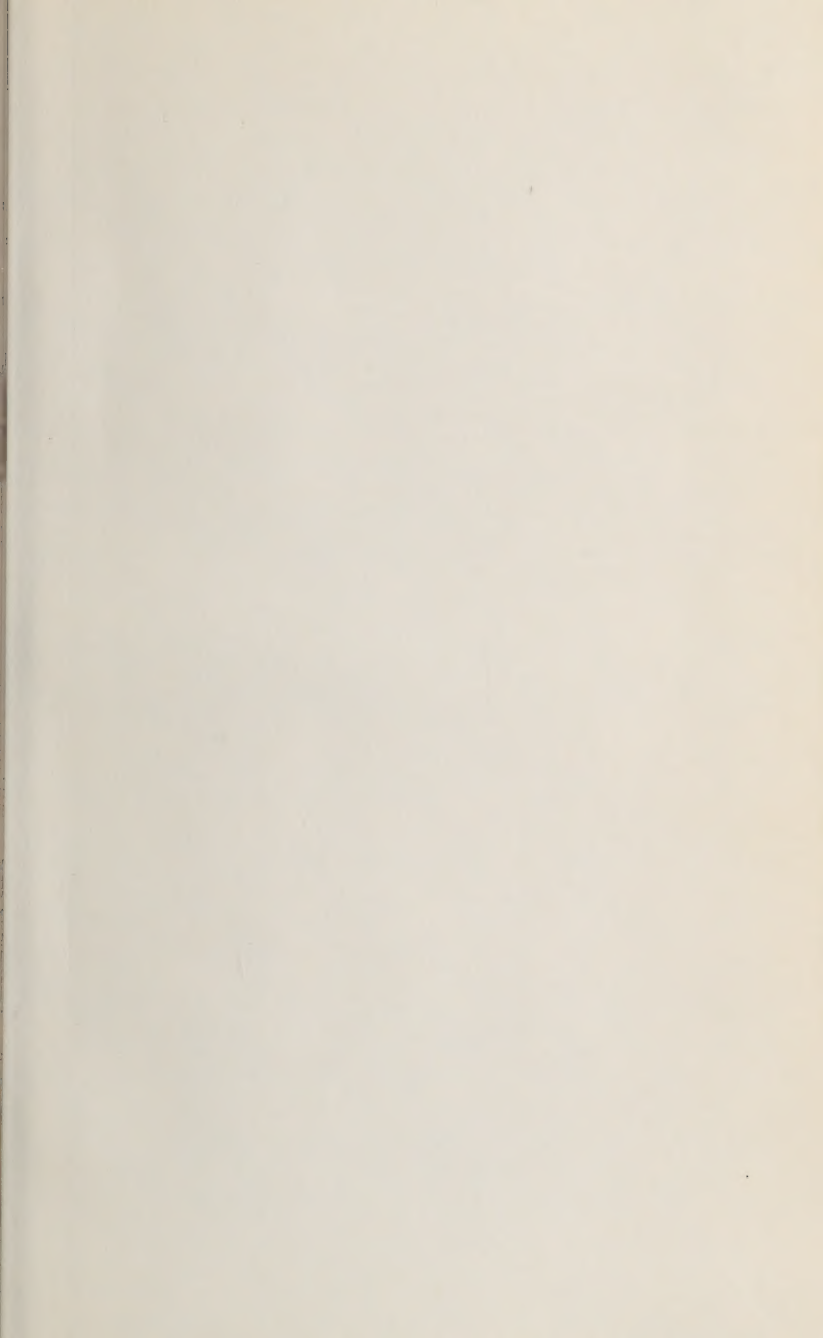
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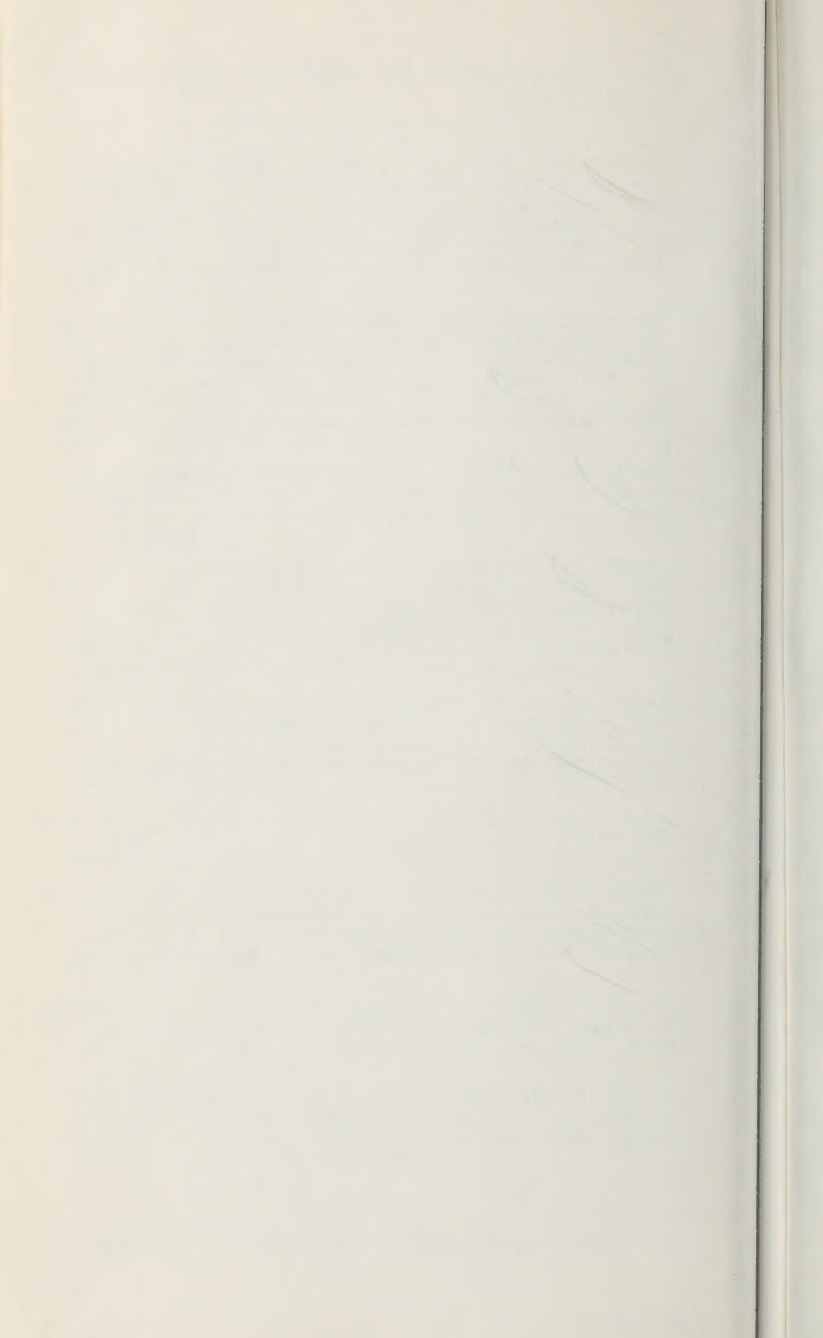
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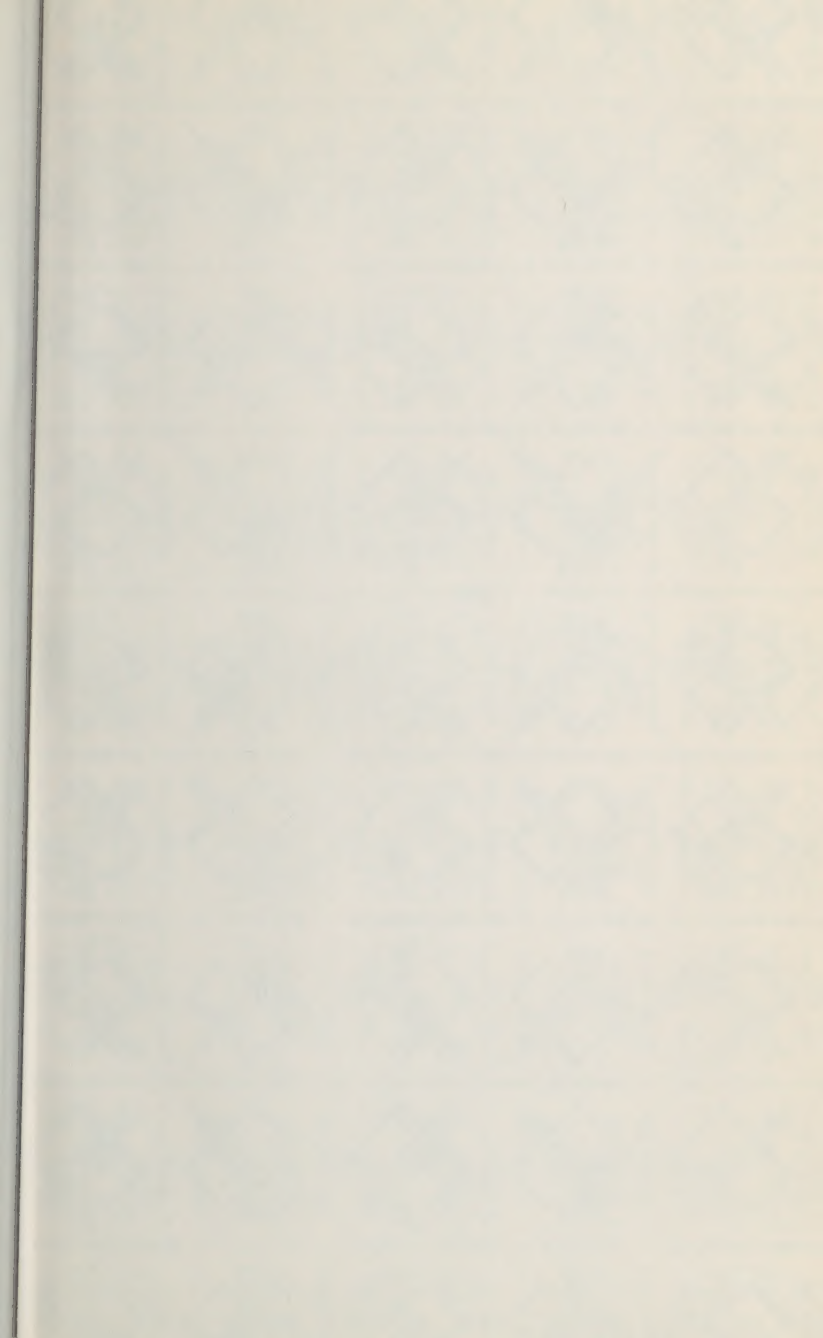
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